Mycobacterium tuberculosis borderline *rpoB* mutations: emerging from the unknown

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To the Editor:

Rifampicin drives the efficacy of the current first-line treatment regimen for tuberculosis (TB) [1]. Mutations in the *rpoB* gene cause rifampicin resistance (RR) of varying levels. Common mutations typically confer high-level, "high-confidence" resistance, providing a selective advantage to *Mycobacterium tuberculosis* during treatment at low fitness cost [2]. Growth-based phenotypic drug-susceptibility testing (pDST) is very reliable for high-confidence mutations. Mutations conferring low-level resistance at high fitness cost are easily lost during primary culture or will cause phenotypically false-susceptible results if not given enough time for growth, especially with the widely used automated MGIT 960 DST [3]. Due to disagreement on their significance, such *rpoB* mutations were called "disputed".

A 2021 World Health Organization (WHO) report concluded that disputed *rpoB* mutations are clinically relevant and recommended to call them "borderline". The report also recommended a lower critical concentration for rifampicin on 7H10 and MGIT media, but not on Löwenstein–Jensen. This will reduce, but not eliminate, pDST misclassification of borderline mutations in the future. The scarcity of outcome data was felt as a major problem [4].

In this paper, we provide information on the adverse impact on treatment outcome in patients with TB showing either a high-confidence or borderline RR mutation and treated with either a first-line rifampicin-throughout or second-line gatifloxacin-based regimen. This retrospective study uses data from the Bangladesh TB control programme, implemented by Damien Foundation (DF) in 13 districts. Before rapid genotypic DST (gDST) became available, new and retreatment patients were treated with the first-line rifampicin-throughout WHO regimens, 6-month category 1 (Cat1) and 8-month category 2 (Cat2). When RR-TB was diagnosed, patients started the second-line gatifloxacin-based shorter treatment regimen [5].

We linked a reference laboratory database of sputum samples from patients consecutively registered between 1995 and 2015 [6] with databases showing data on individual first- or second-line treatment courses. The study protocol was approved by the institutional review board of the ITM (1233/88), Antwerp, Belgium; since it concerned anonymous routinely collected data, the requirement for informed consent was waived. Only outcomes with known *rpoB* result at start of treatment were analysed, but not treatment courses for which baseline samples were collected because of recurrence (failure or relapse) outcome. Mutations 430Pro, 435Tyr, 441Leu (absent in our database), 445Asn, 445Leu, 452Pro and 491Phe were grouped as borderline [4].

Outcomes followed closely those of WHO [7]. First-line treatment relapse follow-up was passive with continuous updating of patient databases when recurrence occurred. Second-line treatment relapse identification relied on 24-month active post-treatment follow-up [5]. Favourable outcome was defined as relapse-free success, adverse outcomes included failure, relapse, and death.

rpoB sequence data showed wild-type *rpoB* for 1547 treatment courses, and a mutation for 669 different treatment courses. Of 669, 26 were excluded from the analysis because the level of resistance was unclear, as more than one *rpoB* mutation was found. Of the remaining 643, 259 corresponded with a first or second rifampicin-throughout treatment course and 384 with second-line gatifloxacin-based courses.

Shareable abstract (@ERSpublications)
Despite their name, borderline *rpoB* mutations are correlated with unfavourable outcomes when rifampicin-throughout treatment is used. They may become the drivers of rifampicin-resistant tuberculosis. Second-line treatment is recommended. https://bit.ly/3nbkZjt

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For wild-type *rpoB*, 94.1% (1455/1547; 95% CI 92.8–95.2%) were cured relapse-free, while 5.9% (92/1547; 95% CI 4.8–7.2%) experienced an adverse outcome (1.9% (n=30) with treatment failure, 1.0% (n=16) with relapse, and 3.0% (n=46) died).

Of 259 patients with TB with an *rpoB* mutation and treated with a first-line regimen, 63 (24.3%, 95% CI 19.2–30.0%) had TB with a borderline mutant. Table 1 shows that relapse-free success was similar among those with a borderline (19/63; 30.2%, 95% CI 19.2–43.0%) or high-confidence mutation (56/196; 28.6%, 95% CI 22.4–35.4%). TB with a 445Asn or 491Phe borderline mutant tended to have non-significant better outcome than with a high confidence mutation (OR 0.34, 95% CI 0.10–1.10).

Of 384 patients treated with a second-line RR-TB regimen, relapse-free success was similar (p=0.8, chi-squared test) among those with a borderline (45/54; 88.9%, 95% CI 77.4–95.8%) or high-confidence mutation (289/330; 87.6%, 95% CI 83.5–90.9).

A recent study on 28 patients with MGIT rifampicin-susceptible but Xpert MTB/RIF-resistant TB showed unusual mutations (DEL428_429, 430Pro, 435Tyr, 441Leu, 445Asn/Cys/Gly/Leu and 452Pro) in all but two, with 81% recurrence on Cat2 [8]. Our study confirms that first-line rifampicin-throughout treatment of TB with a high-confidence or borderline *rpoB* mutation has an equally poor prognosis. When treated with second-line gatifloxacin-based treatment, outcomes were almost as good as those with wild-type rpoB and treated with rifampicin-throughout treatment [1].

The proportion of RR-TB caused by borderline mutations may be important, dependent on the geographical area. Our Antwerp reference laboratory commonly detects proportions between 20 and 30% among new cases from random drug resistance surveys in TB endemic countries. Our best estimate is 26.9% (25/93; 95% CI 18.9–36.7%) for Pakistan in 2013, performing Xpert MTB/RIF from sputum and pDST in parallel (unpublished data).

Borderline *rpoB* mutations are thus the main cause of discordant gDST-resistant/pDST-susceptible results, particularly with MGIT [3]. pDST can miss less fit mutations when overgrown by the more fit wild-type.

TABLE 1 Outcome of rifampicin-throughout treatment by resistance level and type of *rpoB* mutation

	Relapse-free success n	Clinically adverse[#]		OR	95% CI	Bacteriologically adverse[¶]		OR	95% CI
		n	%			n	%		
***rpoB* mutation group**[§]	75	184	71			163	68.5		
High-level	56	140	71.4	1		125	69.1	1	
Borderline (any)	19	44	69.8	0.93	0.50–1.72	38	66.7	0.90	0.48–1.69
Individual borderline mutations[§]									
Leu430Pro	5	13	72.2	0.99	0.35–2.79	11	68.8	0.94	0.32–2.73
Asp435Tyr	2	5	71.4	0.88	0.19–4.07	5	71.4	0.99	0.21–4.56
His445Asn	3	3	50	0.4	0.09–1.83	3	50	0.45	0.10–2.05
His445Leu	1	8	88.9	2.28	0.39–13.29	6	85.7	1.95	0.32–11.84
Leu452Pro	5	13	72.2	0.99	0.35–2.79	11	68.8	0.94	0.32–2.73
Ile491Phe	3	2	40	0.29	0.06–1.50	2	40	0.32	0.06–1.68
Regrouping within borderline *rpoB* mutations[+]									
His445Asn/Ile491Phe	6	5	45.5	0.34	0.10–1.10	5	45.5	0.38	0.12–1.24
Other borderline	13	39	75	1.18	0.59–2.35	33	71.7	1.12	0.55–2.26

Odds ratios calculated using logistic regression. [#]: either failure, relapse, or death (clinically adverse outcomes); % expressed at % of adverse outcomes among patients with relapse-free cure or a clinically adverse outcome. [¶]: either failure or relapse (recurrence: bacteriologically adverse outcome); % expressed at % of adverse outcomes among patients with relapse-free cure or a bacteriologically adverse outcome. Smear-defined outcomes were those of the Bangladesh National TB Programme that closely followed those of the World Health Organization (WHO) [7]. Treatment failure included first-line treatment courses interrupted to switch to second-line treatment. [§]: at the WHO Supranational TB Reference Laboratory (SRL) in Antwerp, *rpoB* sequencing used the Sanger technique with extended primers covering also the 170Phe and 491Phe resistance mutations outside the 81 bp rifampicin resistance determining region (RRDR; codons 426 to 452 in the revised MTB numbering system) [2] or whole genome sequencing (WGS) analysis of the same *rpoB* codons. Non-synonymous mutations were grouped as high-confidence *versus* disputed. Mutations 430Pro, 435Tyr, 441Leu (absent in our database), 445Asn, 445Leu, 452Pro and 491Phe were grouped as disputed [4]. Double mutations were excluded as unclassifiable. Using logistic regression, high-level mutations were used as reference group. [+]: as the proportion of clinically adverse outcomes was lower for 445Asn and 491Phe these mutations were grouped together. Using logistic regression, high-level mutations were used as reference group.

Missed on pDST, KwaZulu-Natal's deadly outbreak of multidrug-resistant (MDR)-TB with fluoroquinolone and second-line injectable-resistance was caused by bacilli with the borderline 452Pro plus 435Gly as double mutation [9]. In Surinam, missed or undertreated borderline 435Tyr caused all documented RR-TB, rising to 12% between 2012 and 2018, the highest prevalence in the Americas [10]. In Sao Paulo State 2014–2017, over half of 283 Xpert RR patients' isolates were MGIT discordant. Around 30% of RR-TB was caused by recent transmission of 445Asn mutated bacilli, virtually all in the city and Metropolitan area [11].

Borderline mutations may also be missed by both Xpert MTB/RIF and LPA (GenoType MTBDRplus version 2.0). Both rapid gDST tests intrinsically miss mutations outside the rifampicin resistance determining region (RRDR), 170Phe and borderline mutation 491Phe. In Eswatini, bacilli carrying the 491Phe mutation, missed first on MGIT and then also on rapid gDST are now responsible for more than half of all RR [12]. Moreover, Xpert MTB/RIF has difficulty to detect some relatively frequent borderline mutations, *i.e.* 435Tyr and 452Pro, that do not cause a complete probe drop-out [13]. Also, heteroresistance (co-presence of mutant and wild-type DNA) is missed often by Xpert MTB/RIF [14], and shows weaker wild-type bands without mutation band on LPA, which is very difficult to identify.

Missed borderline RR on either pDST or rapid gDST has direct epidemiological impact. Under-diagnosis of borderline mutations followed by inadequate treatment, with repeated relapse leading to prolonged and recurrent periods of infectiousness, causes more secondary cases with borderline mutations through "diagnostic selective pressure". In Surinam and Eswatini, despite reportedly high cure rates with first-line treatment, borderline-resistant strains became the main driver of RR transmission [10, 12]. With continuing multiplication, even during rifampicin treatment, genetically enhanced clones may be selected, with an acquired higher level resistance through a second *rpoB* mutation and/or resistance to other drugs added to the regimen [9].

Missed RR may have a grave effect in patients diagnosed with isoniazid-resistant/false rifampicin-susceptible TB. WHO now recommends to strengthen first-line drugs with levofloxacin for isoniazid-resistant but (rapid) gDST proven rifampicin- and fluoroquinolone-susceptible TB [15]. We reported elsewhere that the perceived adverse effect of isoniazid resistance on first-line treatment is mainly due to missed RR on pDST [1], virtually always a borderline mutation. This is in line with the findings of the original TB short-course chemotherapy randomised clinical trials, *i.e.* that only rifampicin but not isoniazid causes excess recurrence [16]. For those patients with missed RR-TB due to borderline mutations, or because rifampicin DST simply could not be performed, *e.g.* because rifampicin DST was not accessible or not operational, acquired fluoroquinolone resistance on top of MDR-TB is expected to emerge and spread rapidly as an unintended but grave consequence of this recommendation [15].

In conclusion, borderline *rpoB* mutations are not that uncommon, depending upon the geographical setting, and responsible for an important part of all clinically, as well as epidemiologically, relevant RR. The term borderline illustrates diagnostic challenges, but not their clinical relevance. As a group, they have the same clinical impact as the high-confidence mutations and should be treated with a second-line regimen.

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Data sharing statement: Deidentified individual participant data and a data dictionary, defining each field in the set, will be made available to others by the corresponding author after approval of the purpose of their use.

Author contributions: A. Van Deun conceived the study. A. Van Deun, K.J.M. Aung, M.A. Hossain and M. Gumusboga collected and prepared the Damien Foundation Bangladesh data. M.A. Hossain, M. Gumusboga and W.B. De Rijk conducted drug susceptibility testing. T. Decroo and A. Van Deun analysed the data. All co-authors contributed to the interpretation of the findings. A. Van Deun and T. Decroo wrote the first draft. All co-authors critically revised the subsequent versions and also approved the final version.

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References

1 Van Deun A, Decroo T, Kya Jai Maug A, *et al.* The perceived impact of isoniazid resistance on outcome of first-line rifampicin-throughout regimens is largely due to missed rifampicin resistance. *PLoS One* 2020; 15: e0233500.

2 Gagneux S, Long CD, Small PM, *et al.* The competitive cost of antibiotic resistance in *Mycobacterium tuberculosis*. *Science* 2006; 312: 1944–1946.

3 Van Deun A, Barrera L, Bastian I, *et al. Mycobacterium tuberculosis* strains with highly discordant rifampin susceptibility test results. *J Clin Microbiol* 2009; 47: 3501–3506.

4 World Health Organization. Technical Report on Critical Concentrations for Drug Susceptibility Testing of Isoniazid and the Rifamycins (Rifampicin, Rifabutin and Rifapentine). 2021. https://apps.who.int/iris/rest/bitstreams/1330649/retrieve Date last accessed: 15 January 2021.

5 Aung KJM, Van Deun A, Declercq E, *et al.* Successful '9-month Bangladesh regimen' for multidrug-resistant tuberculosis among over 500 consecutive patients. *Int J Tuberc Lung Dis* 2014; 18: 1180–1187.

6 Van Deun A, Aung KJM, Hossain MA, *et al.* Twenty years of rifampicin resistance surveillance in Bangladesh: periodic vs. continuous monitoring. *Int J Tuberc Lung Dis* 2018; 22: 1450–1461.

7 World Health Organization. Definitions and Reporting Framework for Tuberculosis – 2013 Revision (Updated December 2014 and January 2020). 2013. http://apps.who.int/iris/bitstream/10665/79199/1/9789241505345_eng.pdf?ua=1 Date last accessed: 13 April 2021.

8 Hu P, Zhang H, Fleming J, *et al.* Retrospective analysis of false-positive and disputed rifampin resistance Xpert MTB/RIF assay results in clinical samples from a referral hospital in Hunan, China. *J Clin Microbiol* 2019; 57: e01707-18.

9 Brown TS, Challagundla L, Baugh EH, *et al.* Pre-detection history of extensively drug-resistant tuberculosis in KwaZulu-Natal, South Africa. *Proc Natl Acad Sci USA* 2019; 116: 23284–23291.

10 Gopie FA, Commiesie E, Baldi S, *et al.* Should treatment of low-level rifampicin mono-resistant tuberculosis be different? *J Clin Tuberc Other Mycobact Dis* 2021; 23: 100222.

11 Brandao AP, Pinhata JMW, Simonsen V, *et al.* Transmission of *Mycobacterium tuberculosis* presenting unusually high discordance between genotypic and phenotypic resistance to rifampicin in an endemic tuberculosis setting. *Tuberculosis (Edinb)* 2020; 125: 102004.

12 Beckert P, Sanchez-Padilla E, Merker M, *et al.* MDR *M. tuberculosis* outbreak clone in Eswatini missed by Xpert has elevated bedaquiline resistance dated to the pre-treatment era. *Genome Med* 2020; 12: 104.

13 Somoskovi A, Deggim V, Ciardo D, *et al.* Diagnostic implications of inconsistent results obtained with the Xpert MTB/RIF assay in detection of *Mycobacterium tuberculosis* isolates with an *rpoB* mutation associated with low-level rifampin resistance. *J Clin Microbiol* 2013; 51: 3127–3129.

14 Blakemore R, Story E, Helb D, *et al.* Evaluation of the analytical performance of the Xpert MTB/RIF assay. *J Clin Microbiol* 2010; 48: 2495–2501.

15 World Health Organization. WHO Treatment Guidelines for Isoniazid-resistant Tuberculosis. Supplement to the WHO Treatment Guidelines for Drug-resistant Tuberculosis. World Health Organization Document 2018; WHO/CDS/TB/2018.7: 1–31. http://apps.who.int/iris/bitstream/10665/260494/1/9789241550079-eng.pdf?ua=1 Date last accessed: 15 January 2021.

16 Fox W, Ellard GA, Mitchison DA. Studies on the treatment of tuberculosis undertaken by the British Medical Research Council Tuberculosis Units, 1946–1986, with relevant subsequent publications. *Int J Tuberc Lung Dis* 1999; 3: S231–SS79.