The potential use of rifabutin for treatment of patients diagnosed with rifampicin-resistant tuberculosis

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Background: Use of the Xpert MTB/RIF assay has increased the number of people diagnosed with rifampicin-resistant tuberculosis (RR-TB), especially in South Africa where Xpert is now the initial diagnostic for individuals with TB symptoms. We hypothesized that a proportion of RR-TB patients determined by Xpert can be treated with a rifabutin-containing regimen.

Methods: Rifabutin susceptibility by *rpoB* mutation was assessed in 349 individuals from South Africa and 172 from Belgium. *rpoB* polymorphisms were identified by Sanger sequencing. Rifampicin and rifabutin susceptibility was assessed phenotypically. A systematic review was performed to comprehensively collate information on rifabutin susceptibility by *rpoB* polymorphism. Rifabutin susceptibility was assigned to *rpoB* polymorphisms based on their positive likelihood ratios and ORs.

Results: One hundred and twelve *rpoB* polymorphisms (67.9% from literature) were identified from all 2045 RR-TB patients, of which 17 polymorphisms could be classified as susceptible/resistant to rifabutin. Eleven polymorphisms were associated with rifabutin susceptibility. The 516GTC mutation was the most common, representing 70% (South Africa) and 76% (Belgium) of all rifabutin-susceptible isolates. At a population level, the 11 polymorphisms associated with rifabutin susceptibility occurred in 33.2% and 16.6% of all South African and Belgian patients diagnosed with RR-TB, respectively.

Conclusions: Identification of the exact *rpoB* polymorphism leading to the diagnosis of RR-TB has the potential to allow inclusion of rifabutin in the treatment regimen of a substantial proportion of RR-TB patients. A randomized controlled trial evaluating the efficacy of a rifabutin-containing TB treatment regimen in these selected patients is needed to provide the evidence required for a change in policy.

Introduction

The WHO estimates that there were 490 000 new cases of MDR tuberculosis (MDR-TB), and an additional 110 000 cases of rifampicin-resistant TB (RR-TB) in 2016.¹ Rapid diagnosis and adequate treatment of drug-resistant TB is essential to optimize treatment outcomes and to prevent transmission of drug-resistant strains. Since the endorsement of the Xpert MTB/RIF assay (Cepheid Inc., Sunnyvale, CA, USA) by the WHO in 2011, >16

million tests have been performed in 122 countries and detection of RR-TB has increased 3–8-fold.²

Rifampicin is a potent bactericidal drug and plays a crucial role in the treatment of drug-susceptible TB due to its sterilizing activity and its ability to penetrate the caseum in sufficient concentrations to kill the bacilli.^{3–5} Rifampicin's importance was recently highlighted in a study that demonstrated that it is the only anti-TB drug that eradicated bacilli within a caseum or granuloma.⁶ Other rifamycins, such as rifabutin and rifapentine, have attributes similar to

those of rifampicin.^{7–11} Currently, rifabutin is used for treatment of drug-susceptible TB in people living with HIV who receive a PI-containing ART regimen because compared with rifampicin it has limited drug–drug interactions with PIs.^{4,12} Rifapentine is mainly used in the treatment of latent *Mycobacterium tuberculosis* (MTB) infection.¹³

RR-TB requires prolonged treatment with drugs that are less efficient and more toxic.^{14,15} Resistance to rifampicin in MTB is strongly associated with mutations in the rifampicin resistance-determining region (RRDR), an 81 bp region within the *rpoB* gene.^{16–18} Despite cross-resistance amongst rifamycins, rifabutin has been shown to retain activity against some MTB strains, especially those with mutations in codons 516 and 533.^{11,19–22} We hypothesize that rifabutin can be used to treat a substantial proportion of patients with RR-TB diagnosed by the Xpert MTB/RIF assay. To inform the targeted use of rifabutin for RR-TB, we aimed to comprehensively assess the association between rifabutin drug susceptibility and genetic polymorphisms in the *rpoB* gene.

Materials and methods

Selection of clinical *M. tuberculosis* isolates

We used three sources of clinical MTB isolates: two sets of population-representative samples and one large MTB culture bank. One set of samples were MTB isolates collected as part of a prospective cohort study ('EXIT-RIF') aimed at comparing the outcome of patients diagnosed with RR-TB by MTBDRplus (Hain Lifescience) or Xpert MTB/RIF between November 2012 and December 2013 in three South African provinces (Free State, Eastern Cape and Gauteng). Pre-treatment isolates sent to the South African Medical Research Council Centre for Tuberculosis Research (SAMRC-CTR) at the University of Stellenbosch were eligible for inclusion in this analysis. The second set of clinical MTB isolates consisted of all RR-TB patients diagnosed in Belgium between 2004 and 2016 who had information available on rifabutin resistance. Lastly, the MTB databank of the SAMRC-CTR, which consists of ~40 000 drug-resistant MTB isolates collected in the Western Cape province since 2001, was queried to identify isolates containing mutations in the *rpoB* gene that were not present in the EXIT-RIF or Belgian sample sets.

Targeted DNA sequencing of the RRDR of the *rpoB* gene

At the SAMRC-CTR, the RRDR of the *rpoB* gene was amplified using a forward primer (5'-TGGTCCGCTTGACGAGGGTCAGA-3') and a reverse primer (5'-CTCAGGGTTTCGATCGGGCACAT-3') designed to amplify a product of 437 bp as described previously.²³ Amplification was confirmed by the detection of a fluorescent melting curve signature in the presence of Syto-9 (Molecular Probes, Eugene, OR, USA) by high-resolution melting analysis. The DNA sequencing reaction was done on a GeneAmp 9700 thermal cycler (Applied Biosystems, Foster City, CA, USA) using the BigDye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Austin, TX, USA). The products were electrophoresed on an ABI 3730xl Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and sequences were analysed on the BioEdit sequence alignment editor (<http://www.mbio.ncsu.edu/bioedit/bioedit.html>) with H37Rv as the reference strain.

The strains collected from patients diagnosed with RR-TB in Belgium were processed at the Scientific Institute of Public Health (Brussels, Belgium). The *rpoB* sequencing was performed as described previously by Kapur *et al.*²⁴ and the sequences were analysed using Blast2seq (NCBI) with H37Rv as the reference strain.

Phenotypic MIC determination and drug susceptibility testing of rifampicin and rifabutin

MICs of rifampicin and rifabutin were determined for all EXIT-RIF samples and the samples selected from the MTB databank. If multiple isolates with the same *rpoB* polymorphism were available, then up to four isolates were evaluated. All MICs were performed using the non-radiometric BACTECTM MGIT 960 method (BD Diagnostic Systems, NJ, USA) as previously described.²⁵ The rifamycin drugs (Sigma-Aldrich, South Africa) were dissolved in 100% DMSO to obtain stock solutions. The MICs were determined using 100, 50, 20, 10, 5 and 1 mg/L rifampicin and 2, 1, 0.5, 0.25 and 0.125 mg/L rifabutin. A fully susceptible MTB laboratory strain H37Rv (ATCC 27294) was included as a control in all MIC assays. A sample with an MIC below or equal to the breakpoint concentration of 1 mg/L for rifampicin and 0.5 mg/L for rifabutin was judged to be susceptible.²⁶

At the Scientific Institute of Public Health in Belgium, phenotypic drug susceptibility tests (DSTs) were performed using the radiometric BACTECTM 460TB system until 2012 and thereafter the BACTECTM MGIT 960 using the protocol described in Cambau *et al.*²⁷ Evaluations at the screening concentrations were performed: 2 mg/L in the BACTECTM 460TB and 1 mg/L in the BACTECTM MGIT 960 system for rifampicin, and 0.5 mg/L in the BACTECTM 460TB and 0.1 mg/L in the BACTECTM MGIT 960 system for rifabutin. MICs were not determined.

Literature search

We performed a systematic literature search to identify published information on rifabutin drug susceptibility by *rpoB* polymorphism. Studies were identified through PubMed, Scopus and Science Direct searches on 6 February 2018 using the MESH terms 'rifabutin' and 'MIC' and '*rpoB*' and 'rifampicin' and '*Mycobacterium tuberculosis*'. All papers were assessed for the following inclusion criteria: studies must report original data, present rifabutin MIC data by *rpoB* polymorphisms, be published in a peer-reviewed journal between 1995 and 2018, and be written in English. We abstracted data on the location of the study population, year of study, *rpoB* polymorphism, rifampicin MIC, rifabutin MIC, technique used to determine rifampicin and rifabutin MICs, concentrations used for MIC testing and number of isolates.

Data analysis

We first identified all unique *rpoB* polymorphisms, summarized the range of MIC results for rifampicin and rifabutin by *rpoB* polymorphism and identified unique combinations of *rpoB* polymorphism and rifampicin MIC. Next, we calculated the frequencies of isolates susceptible and resistant to rifabutin and rifampicin for each of the *rpoB* polymorphisms observed. We applied the recently published standardized methodology for the statistical validation of the association between mutations and phenotypic drug resistance to derive likelihood ratios and ORs with their respective *P* values.²⁸ To account for multiple testing, *P* values were corrected using the Benjamini–Hochberg procedure to control the false discovery rate.²⁹ *rpoB* polymorphisms with a positive likelihood ratio and OR (significant at the 0.05 level) >10, between 5 and ≤10, or between 1 and ≤5 were classified as high, moderate or minimal confidence for association with resistance, respectively. A significant positive likelihood ratio or OR <1 was used for classifying polymorphisms as associated with rifabutin susceptibility. Associations for which statistical significance could not be obtained were classified as indeterminate.

The population prevalence of rifabutin-susceptible isolates among all rifampicin-resistant isolates was determined separately for the Belgian and South African populations of patients diagnosed with RR-TB, the latter by extrapolating our findings to the distribution of *rpoB* polymorphisms in the 349 EXIT-RIF patients with *rpoB* mutation information available.

Table 1. *rpoB* polymorphisms and range of rifampicin and rifabutin MICs in 72 isolates selected from clinical isolates from the EXIT-RIF cohort of 349 patients to represent the unique combinations of *rpoB* polymorphisms and rifampicin MICs observed in this cohort

Codon	Nucleotide change	Amino acid change	MIC range (mg/L)		No. of isolates
			rifampicin	rifabutin	
509	deletion 6 bp	frameshift	>1 to ≤5	<0.125	1
511	CTG→CCG	Leu→Pro	<1	<0.125	2
513	CAA→AAA	Gln→Lys	>100	>2	2
513	CAA→CCA	Gln→Pro	>100	0.5–1.0	2
513	CAA→CTA	Gln→Leu	>100	>2.0	2
515	deletion 6 bp	frameshift	>100	>2.0	2
516	GAC→GCC	Asp→Ala	<1.0	<0.125	2
516	GAC→GTC	Asp→Val	>1 to <50	<0.125–0.5	12
516	GAC→TAC	Asp→Tyr	<1 to >10	<0.125–0.25	3
516	GAC→TTC	Asp→Phe	>1 to <5	<0.125	1
516	GAC→TGC	Asp→Cys	<1.0	<0.125	1
516	deletion 3 bp	frameshift	>1 to <20	0.25–0.5	3
517	deletion 3 bp	frameshift	>1 to <10	<0.125	2
518	deletion 3 bp	frameshift	<1.0	<0.125	1
522	TCG→TTG	Ser→Leu	>1 to <5	<0.125	1
526	CAC→AAC	His→Asn	<1.0	<0.125	2
526	CAC→CGC	His→Arg	>100	>2.0	2
526	CAC→CTC	His→Leu	>1 to >100	<0.125 to >2.0	2
526	CAC→GAC	His→Asp	>100	>2.0	4
526	CAC→TAC	His→Tyr	>100	>2.0	2
526	CAC→TGC	His→Cys	>1 to <5	<0.125	1
531	TCG→TTG	Ser→Leu	>5 to >100	>0.025 to >2.0	10
531	TCG→TTT	Ser→Phe	>100	>2.0	2
533	CTG→CCG	Leu→Pro	<1 to <10	<0.125–1.0	4
511 + 516	CTG→CCG + GAC→TAC	Leu→Pro + Asp→Tyr	>5 to 100	0.5 to >2.0	3
511 + 526	CTG→CCG + CAC→TAC	Leu→Pro + His→Tyr	>1 to <5	<0.125	1
512 + 516	AGC→AGG + GAC→GGC	Ser→Arg + Asp→Gly	>1 to <5	<0.125	1
513 + 516	CAA→GAA + GAC→GTC	Gln→Glu + Asp→Val	>100	>2.0	1

Finally, we describe the treatment outcome of Belgian patients treated with a rifabutin-containing regimen.

Results

Among the 349 TB patients enrolled in the EXIT-RIF study who had a viable isolate sent to the SAMRC-CTR laboratory, 28 different mutations in the RRDR region of the *rpoB* gene were identified (Table 1). Among 72 clinical isolates representing these 28 *rpoB* polymorphisms, 48 unique combinations of *rpoB* polymorphism and rifampicin MIC level were observed.

Among the 193 isolates collected from patients diagnosed with RR-TB in Belgium, 172 had information on *rpoB* polymorphism. Among these 172 isolates, eight different *rpoB* polymorphisms were identified (Table 2). All eight *rpoB* polymorphisms present in the Belgian population were also present in the South African EXIT-RIF population.

From the SAMRC-CTR MTB databank, six additional different *rpoB* polymorphisms not occurring in the EXIT-RIF or Belgian population were identified, representing eight unique combinations of *rpoB* mutations and rifampicin MIC level (Table 3).

The systematic literature search identified 43 unique articles, of which 29 articles were excluded after screening the titles and abstracts, resulting in 13 eligible studies providing information on rifabutin drug susceptibility by *rpoB* polymorphism in 1807 MTB isolates^{7,11,17,18,21,30–37} (Figure 1 and Table S1, available as [Supplementary data](#) at JAC Online). A total of 103 *rpoB* polymorphisms were described in these 1807 isolates, of which 76 were not present in the EXIT-RIF study, Belgian sample collection or the SAMRC-CTR MTB databank.

Taking all information together (two population-representative sample sets, one large MTB databank and a systematic literature review), 112 different *rpoB* polymorphisms associated with rifampicin resistance were identified in 2045 clinical isolates (Table S2). Of these, a statistically significant association between mutation and rifabutin drug resistance could be calculated for 17 *rpoB* polymorphisms; the remainder were classified as indeterminate (Table 4 and Table S3). The 17 polymorphisms were present in 1808 (88.4%) of the 2045 isolates and were detected in codons 511, 513, 516, 522, 526, 531, 533 and 511+515. Of these 17 *rpoB* polymorphisms, 11 were classified as susceptible to rifabutin (511 CTG→CCG, 526 CAC→AAC,

Table 2. *rpoB* polymorphisms and rifampicin and rifabutin drug susceptibility in 172 rifampicin-resistant isolates identified in Belgium between 2004 and 2016

Codon	Nucleotide change	Amino acid change	Susceptibility testing		No. of isolates
			rifampicin	rifabutin	
513	CAA→AAA	Gln→Lys	resistant	resistant	1
516	GAC→GTC	Asp→Val	resistant	resistant/susceptible	19
516	GAC→TAC	Asp→Tyr	resistant	susceptible	3
526	CAC→CTC	His→Leu	resistant	resistant/susceptible	3
526	CAC→GAC	His→Asp	resistant	resistant	5
526	CAC→TAC	His→Tyr	resistant	resistant	4
531	TCG→TTG	Ser→Leu	resistant	resistant/susceptible	114
531	TCG→TGG	Ser→Trp	resistant	resistant	2

Table 3. *rpoB* polymorphisms and rifampicin and rifabutin MICs of isolates selected from the SAMRC-CTR MTB databank

Codon	Nucleotide change	Amino acid change	MIC (mg/L)		No. of isolates
			rifampicin	rifabutin	
513	CAA→AAA	Gln→Lys	>100	>2.0	1
516	GAC→GGC	Asp→Gly	<1.0	<0.125	1
517	deletion 9 bp	frameshift	<1.0	<0.125	2
525	deletion 9 bp	frameshift	>20 to <50	>2.0	2
526	CAC→AGC	His→Ser	>1 < 5	<0.125	1
526	CAC→GGC	His→Gly	<1.0	<0.125	2
531	TCG→TGG	Ser→Trp	>100	>2.0	4
533	CTG→GAG	Leu→Glu	<1.0	<0.125	1
516 + 533	GAC→GGC + CTG→CCG	Asp→Gly + Leu→Pro	>50 to <100	>1 to <2	1

Figure 1. Flow diagram of systematic literature review performed on 6 February 2018 for articles published from 1995–2018.

516 GAC→GTC, 516 GAC→TTC, 516 GAC→TAC, 522 TCG→TTG, 526 CAC→CTC, 526 CAC→CTC, 533 CTG→CCG, 511+515 CTG→CCG + ATG→GTG and 511+515 CTG→CCG + ATG→GTG). Four *rpoB* polymorphisms were classified as resistant to rifabutin with high confidence (513 CAA→AAA, 526 CAC→CGC, 531 TCG→TGG and 531 TCG→TTG), one as resistant to rifabutin with moderate confidence (526 CAC→GAC) and one as resistant to rifabutin with minimal confidence (526 CAC→TAC).

Table 4. Proportion of rifabutin-resistant samples by *rpoB* polymorphisms for which enough information was available to reach a conclusion

Codon	Nucleotide change	Amino acid change	No. of samples	No. RBT resistant	OR	OR 95% CI	LR+	LR+ 95% CI	P value		RBT susceptibility
									unadjusted	corrected	
511	CTG→CCG	Leu→Pro	16	0	0.00	0.00	0.00	0.15	<0.01	<0.01	susceptible
513	CAA→AAA	Gln→Lys	25	25	inf	1.77	0.85	inf	<0.01	0.02	high confidence, resistant
516	GAC→GTC	Asp→Val	173	15	0.02	0.01	0.03	0.04	<0.01	<0.01	susceptible
	GAC→TAC	Asp→Tyr	37	0	0.00	0.00	0.00	0.06	<0.01	<0.01	susceptible
	GAC→TCC	Asp→Ser	4	0	0.00	0.00	0.00	0.66	<0.01	0.01	susceptible
	GAC→TTC	Asp→Phe	10	0	0.00	0.00	0.00	0.24	<0.01	<0.01	susceptible
522	TCG→TTG	Ser→Leu	15	0	0.00	0.00	0.00	0.16	<0.01	<0.01	susceptible
526	CAC→AAC	His→Asn	15	0	0.00	0.00	0.00	0.16	<0.01	<0.01	susceptible
	CAC→AGC	His→Arg	54	54	inf	4.05	1.86	inf	<0.01	<0.01	high confidence, resistant
	CAC→CTC	His→Leu	35	4	0.03	0.01	0.04	0.10	<0.01	<0.01	susceptible
	CAC→GAC	His→Asp	87	83	6.03	2.25	5.77	15.65	<0.01	<0.01	moderate confidence, resistant
	CAC→GGC	His→Gly	4	0	0.00	0.00	0.00	0.66	<0.01	0.01	susceptible
	CAC→TAC	His→Tyr	111	102	3.30	1.65	3.15	6.18	<0.01	<0.01	minimal confidence, resistant
531	TCG→TGG	Ser→Trp	39	39	inf	2.86	inf	inf	<0.01	<0.01	high confidence, resistant
	TCG→TTG	Ser→Leu	1134	1104	30.74	20.85	10.24	14.48	<0.01	<0.01	high confidence, resistant
533	CTG→CCG	Leu→Pro	43	12	0.10	0.05	0.11	0.21	<0.01	<0.01	susceptible
511 + 515	CTG→CCG + ATG→GTG	Leu→Pro + Met→Val	6	0	0.00	0.00	0.00	0.41	<0.01	<0.01	susceptible

LR+, positive likelihood ratio; inf, infinite; RBT, rifabutin.

The population prevalence of TB patients with an *rpoB* polymorphism-containing isolate (i.e. diagnosed as rifampicin-resistant on Xpert MTB/RIF) who could potentially benefit from a rifabutin-containing regimen was determined for a representative sample of South African (Free State, Gauteng and Eastern Cape) patients and for the cohort of RR-TB patients diagnosed in Belgium between 2004 and 2016 (Figure 2). In South Africa, an estimated 33.2% (95% CI 28.1%–38.6%) of these patients may benefit from a rifabutin-containing regimen. The most common rifabutin-susceptible *rpoB* polymorphism was 516 GAC→GTC, with a population prevalence of 23.2% (95% CI 18.7%–28.3%) and representing 69.7% (95% CI 60.0%–77.9%) of all *rpoB* polymorphism-containing strains susceptible to rifabutin. In Belgium, 16.6% (95% CI 11.5%–23.4%) of all rifampicin-resistant cases may benefit from a rifabutin-containing treatment regimen. Again, the 516 GAC→GTC was the most common *rpoB* polymorphism, with a population prevalence of 12.6% (95% CI 8.2%–18.8%), representing 76.0% (95% CI 56.6%–88.5%) of all rifampicin-resistant strains susceptible to rifabutin.

Data on treatment outcomes of patients with RR-TB who received rifabutin were available for 17 Belgian patients, of whom 5 had pre-XDR and 1 had XDR-TB. Among these, 13 were cured, 3 were transferred out and 1 was lost to follow-up. These outcomes were similar to those achieved in Belgian patients with RR-TB who were also resistant to rifabutin. Among the 13 patients that were cured, 10 had a strain containing an *rpoB* mutation associated with rifabutin susceptibility [516 GAC→GTC ($n=8$), 516 GAC→TAC ($n=1$) or 526 CAC→CTC ($n=1$)].

Discussion

In this study, we comprehensively collated information on rifampicin and rifabutin MIC or DST by *rpoB* polymorphism in over 2000 clinical isolates from 12 countries. We identified 112 different *rpoB* polymorphisms, representing the largest spectrum of *rpoB* polymorphisms with associated phenotypic drug susceptibility results for rifampicin and rifabutin to date. Among these 112 *rpoB* polymorphisms, 11 were susceptible to rifabutin, 6 were resistant to rifabutin (4 high confidence, 1 moderate confidence and 1 minimal confidence); the remaining 95 had insufficient data for classification (i.e. indeterminate for rifabutin). The 11 rifabutin-susceptible *rpoB* polymorphisms had previously been reported as potentially associated with rifabutin susceptibility^{11,31,35,36} but the statistical significance of this association had not yet been assessed. Our results therefore add important data to the growing body of knowledge on genotypic–phenotypic associations in MTB, especially as neither of the two recent landmark studies characterizing the statistical association between mutations in MTB and resistance to TB drugs included rifabutin in their analysis.^{28,38}

Of the polymorphisms classified as susceptible to rifabutin, most have been identified as resistant to rifampicin, highlighting the importance of potential inclusion of rifabutin in the treatment regimen. Miotto *et al.*²⁸ classified four of these *rpoB* polymorphisms as high confidence rifampicin resistant (516 GAC→GTC, 516 GAC→TTC, 526 CAC→CTC and 526 CAC→CTC), three as moderate confidence (516 GAC→TAC, 522 TCG→TTG and 533 CTG→CCG) and two as minimal confidence rifampicin resistant (511 CTG→CCG and 526 CAC→AAC). The remaining two rifabutin-susceptible polymorphisms were classified by Miotto *et al.*²⁸ as

Figure 2. Population prevalence of the eight most prevalent *rpoB* polymorphisms associated with rifabutin susceptibility in rifampicin-resistant populations in South Africa and Belgium. Three additional *rpoB* polymorphisms associated with rifampicin susceptibility (526 CAC→GGC, 516 GAC→TCC and 511+515 CTG→CCG + ATG→GTG) were reported in the literature but did not occur in the Belgian or South African cohorts.

indeterminate for rifampicin (511+515 CTG→CCG + ATG→GTG and 511+515 CTG→CCG + ATG→GTG). The four *rpoB* polymorphisms classified as resistant to rifabutin in our study were all classified as high confidence rifampicin resistant by Miotto et al.²⁸

At population level, the 11 *rpoB* polymorphisms associated with rifabutin susceptibility represented 33.2% (95% CI 28.1%–38.6%) of all South African patients determined as rifampicin resistant by Xpert MTB/RIF and 16.6% (95% CI 11.5%–23.4%) of all Belgian RR-TB patients. The 516 GAC→GTC polymorphism was the most frequent, occurring in 23.2% of South African and 12.6% of Belgian rifampicin-resistant patients, and representing 69.7% and 76.0% of rifabutin-susceptible *rpoB* polymorphisms in South Africa and Belgium, respectively. The 33.2% estimate for South Africa is slightly higher than the estimate of the only other population-based study, which estimated that 27% of 189 rifampicin-resistant cases in South Africa may benefit from the inclusion of rifabutin in their treatment regimen.³³ A recent publication from Malawi suggests that our finding that a substantial proportion of people diagnosed with RR-TB by Xpert MTB/RIF have rifabutin-susceptible TB may be generalizable, as 22% (8/37) of the rifampicin-resistant Malawian isolates contained one of the 11 *rpoB* polymorphisms we found to be associated with rifabutin susceptibility, again with the 516 GAC→GTC polymorphism being the most common (75% of all potentially rifabutin-susceptible isolates).³⁹

Our results are of public health significance as they suggest that one in six patients with RR-TB (a marker of MDR-TB) in Belgium, a low MDR-TB burden country, and one in three patients with RR-TB in South Africa, a high MDR-TB burden country, could potentially benefit from inclusion of rifabutin in their treatment regimen. Implementation of such a strategy would require a rapid diagnostic tool to identify the exact *rpoB* polymorphism that led to the diagnosis of RR-TB. While WGS could identify all 11 rifabutin-susceptible *rpoB* polymorphisms, the most common polymorphism (516 GAC→GTC) can also be detected by the Hain MTBDR_{plus} line probe assay (MUT1 probe⁴⁰) and the Xpert MTB/RIF Ultra assay (based on unique melting temperature analysis of molecular beacons that hybridize to the *rpoB* probe 2).⁴¹

An important strength of this study was the rigorous assessment of rifabutin and rifampicin drug resistance profile by *rpoB* polymorphism and the use of multiple sources (two population-based samples, one large MTB databank and a systematic literature review), resulting in the most comprehensive evaluation of rifabutin susceptibility in rifampicin-resistant clinical isolates to date. Another strength was the inclusion of two population-representative samples of RR-TB patients, one from a high TB burden country (South Africa) and one from a low TB burden country (Belgium), which allowed us to estimate the potential public health relevance of the use of rifabutin in an individualized treatment regimen for patients diagnosed with RR-TB. Our study did suffer from some limitations. First, insufficient data were available to classify 95 (85%) of the 112 *rpoB* polymorphisms identified. This finding is, however, not surprising, as Coll et al.³⁸ also observed that 93% of all polymorphisms in MTB had an allele frequency <1%. Second, we only considered mutations in the RRDR region of the *rpoB* gene and did not include polymorphisms outside this region, in other genes or other mechanisms such as efflux pumps. Finally, in the absence of sufficient data on treatment outcome of rifabutin-containing regimens in selected patients with RR-TB, we can only report on the genotypic–phenotypic associations and cannot infer the effectiveness of such regimens.

In conclusion, identification of *rpoB* polymorphisms associated with rifabutin susceptibility by WGS, Hain MTBDR_{plus} line probe or Xpert MTB/RIF Ultra melting curves have the potential to optimize the treatment of a substantial proportion of RR-TB patients worldwide. A randomized controlled trial evaluating the efficacy of a rifabutin-containing treatment regimen in these selected patients is needed to provide the evidence required for a change in policy.

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Transparency declarations

None to declare.

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Supplementary data

Tables S1 to S3 appear as [Supplementary data](#) at JAC Online.

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