Utilization of molecular resistance test results as tools to support public health efforts for improved control of rifampicin-resistant tuberculosis

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Publication date
2020

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Chapter 3

Xpert Ultra can unambiguously identify specific rifampicin resistance-conferring mutations

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Journal of Clinical Microbiology 2018, 56:e00686-18. https://pubmed.ncbi.nlm.nih.gov/29925643

INTRODUCTION

The deluge of data produced by XpertMTB/RIF (Cepheid) can help improve global rifampicin-resistant tuberculosis (RR-TB) control strategies through molecular epidemiological surveillance (1, 2). Recently, a new version of the test – Xpert Ultra (hereinafter called Ultra) was released (3). Determining the relationship between RR-conferring *rpoB* mutations, Ultra probes, and melting temperature shifts (ΔTm) – the difference between mutant and wildtype melting temperatures – allows Ultra results to be utilized for rapid detection of RR-TB strains and related underlying *rpoB* mutations.

METHODS

To validate the usefulness of Ultra results for predicting specific mutations, we analyzed 10 RS-TB and 107 RR-TB strains from the Belgian Coordinated Collections of Microorganisms in the Institute of Tropical Medicine, Antwerp, Belgium. These strains harbor 36 unique RR-conferring mutations determined by *rpoB* sequencing.

RESULTS

Overall, 31/32 (97%) mutations inside the Rifampicin Resistance Determining Region (RRDR) were correctly identified by Ultra. Of concern, mutation His445Arg gave a "RIF Resistance INDERTERMINATE" result among 3/4 strains tested while it was reported as RR in the initial validation study (3). The silent mutation Thr444Thr was not reported as RR (Figure 1). The RR-conferring mutations on codons 170, 250, 299, 482, and 491 situated outside the RRDR were not detected.

The probe reactions observed were largely in agreement with previous results (3) albeit we noted that mutations Met434Val, Met434Thr and those in codon 435 were captured only by probe rpoB2; Ser450Leu and Ser450Trp were captured by both probes rpoB3 and rpoB4a, His445Arg was captured only by probe rpoB3; and Lys446Gln was captured only by probe rpoB4.

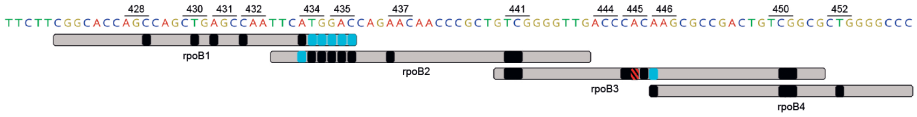

Figure 1. Overview of Xpert Ultra test results. The observed probe reactions for each RRDR mutation were laid over the claimed probe coverage (light gray). Shown in black are probe reactions concordant with manufacturer claims, in blue are probe reactions missed by one probe but captured by another probe, and in red is a probe reaction representing a "RIF Resistance Indeterminate" result from 3 out of 4 strains tested. Results in the hatched pattern were superimposed for greater visibility.

All mutations except those in codon 450 were associated with a negative ΔTm (Figure 2). The combination of ΔTm values with the capturing probes enabled to differentiate mutations in codons 428, 430, 431, 432, 434, 435, 441, 445, 446, and 452, including disputed mutations (4) (Table 1). Mutation Asp435Tyr was unambiguously distinguished from Asp435Val through probe rpoB2|ΔTm|, while mutations Ser441Gln and Ser441Leu were discriminated from the rest by |ΔTm| values of probes rpoB2 and rpoB3. Mutations His445Asp and His445Tyr were distinguished from disputed mutations His445Leu and His445Asn through probe rpoB3 |ΔTm|. Ser450Leu was distinguished from Ser450Trp by probe rpoB4A |ΔTm| except for one strain with an outlier rpoB4A Tm of 70.9°C in contrast to the other 13 strains with rpoB4A Tm of 73.3-73.8°C. The indeterminate result associated with His445Arg may be caused by its |ΔTm|=1.8°C compared with |ΔTm| typically exceeding 2°C for other mutations.

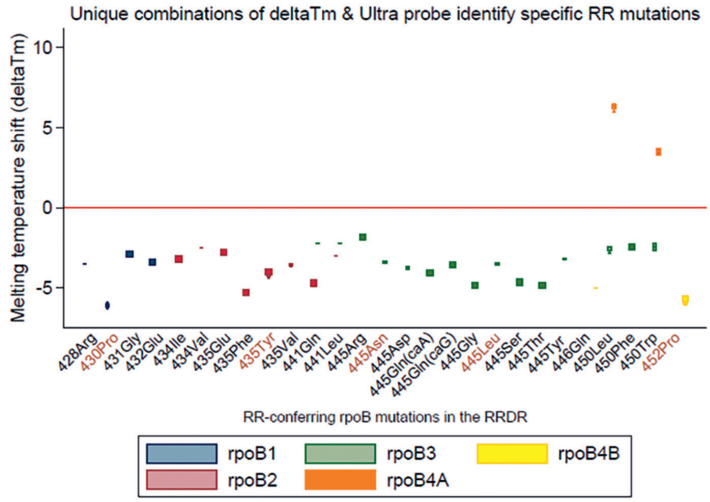

Figure 2. Melting temperature shifts (ΔTms) observed upon detection of a rifampin resistance (RR)-conferring rpoB mutation in the RR-determining region (RRDR) by Xpert Ultra. The y axis reflects the melting temperature difference (ΔTm) between mutant and wild-type probe-amplicon hybrids, while the x axis shows the mutations that we tested. The data points on the graph are ΔTm values grouped by their associated Ultra probes (differentiated by color), which correspond to a specific rpoB mutation. x axis labels in brown are disputed mutations.

CONCLUSIONS

Our findings confirm the ability of Ultra to unambiguously identify a wide range of RRDR mutations. With the unprecedented roll-out of XpertMTB/RIF and associated connectivity solutions, such as DataToCare (Savics, Belgium) and GXAlert (SystemOne, USA) (2), Ultra results may be exploited to rule-out transmission between RR-TB patients in a specific setting, distinguish relapse from reinfection, and resolve discordance between an RR Ultra result and a low-level RS phenotypic result due to a disputed mutation. For such applications, it is key that ΔTm values are included in the exported results.

Acknowledgement

This work was supported by Erasmus Mundus Joint Doctorate Fellowship grant 2016-1346 to KCSN.

Table 1. Xpert Ultra raw results[a]

Mutation(s)[b]	No. of strains tested	Nucleotide change(s)	Xpert Ultra probe(s)	Wild-type T_m range(s) (mean[s])	Mutant T_m range(s)	$\lvert\Delta T_m\rvert$ mean(s) or range(s)
Val170Phe	3	GTC→TTC	ND	ND	ND	ND
Glu250Gly#	2	GAG→GGG	ND	ND	ND	ND
Arg299Cys#	1	CGC→TGC	ND	ND	ND	ND
**Leu430Pro*	8	CTG→CCG	rpoB1	69.1–69.5 (69.3)	63.0–63.4	5.9–6.3
Leu430Pro + *Met434Ile	1	CTG→CCG; ATG→ATA	rpoB1; rpoB2	69.1–69.5 (69.3); 72.8–73.2 (73)	63.2; 69.8	6.1; 3.2
Leu430Pro + Met434Val	1	CTG→CCG; ATG→GTG	rpoB1	69.1–69.5 (69.3)	63.0	6.3
Leu430Pro + His445Gln	1	CTG→CCG; CAC→CAG	rpoB1; rpoB3	69.1–69.5 (69.3); 75.5–76.0 (75.75)	63.5; 72.2	5.8; 3.6
Leu430Pro + His445Gln	1	CTG→CCG; CAC→CAA	rpoB1; rpoB3	69.1–69.5 (69.3); 75.5–76.0 (75.75)	63.1; 71.7	6.2; 4.1
Asp435Gly + Met434Thr	1	GAC→GGC; ATG→ACG	rpoB2	72.8–73.2 (73)	69.7	3.3
*Asp435Phe	1	GAC→TTC	rpoB2	72.8–73.2 (73)	67.7	5.3
**Asp435Tyr*	11	GAC→TAC	rpoB2	72.8–73.2 (73)	68.6–69.0	4.0–4.4
Asp435Tyr + Asn437Asp	1	GAC→TAC; AAC→GAC	rpoB2	72.8–73.2 (73)	66.6	6.4
Asp435Tyr + Met434Ile	1	GAC→TAC; ATG→ATT	rpoB2	72.8–73.2 (73)	68.5	4.5
*Asp435Val	5	GAC→GTC	rpoB2	72.8–73.2 (73)	69.3–69.5	3.5–3.7
Asp435Val + Gln432Glu	1	GAC→GTC; CAA→GAA	rpoB2; rpoB1	72.8–73.2 (73); 69.1–69.5 (69.3)	70.5; 65.9	2.5; 3.4
*Ser441Gln	1	TCG→CAG	rpoB2; rpoB3	72.8–73.2 (73); 75.5–76.0 (75.75)	68.3; 73.5	4.7; 2.3
*Ser441Leu	1	TCG→TTG	rpoB2; rpoB3	72.8–73.2 (73); 75.5–76.0 (75.75)	70.0; 73.5	3.0; 2.3
His445Gly	1	CAC→GGC	rpoB3	75.5–76.0 (75.75)	70.9	4.9
His445Thr	1	CAC→ACC	rpoB3	75.5–76.0 (75.75)	70.9	4.9
His445Ser	1	CAC→AGC	rpoB3	75.5–76.0 (75.75)	71.1	4.7
His445Ser + *Lys446Gln + Thr444Thr	1	CAC→TCC; AAG→CAG; ACC→ACG	rpoB4B	67.0–67.6 (67.3)	62.3	5.0
His445Asp	3	CAC→GAC	rpoB3	75.5–76.0 (75.75)	71.9–72.1	3.7–3.9
His445Leu	2	CAC→CTC	rpoB3	75.5–76.0 (75.75)	72.2–72.3	3.5–3.6
His445Asn	2	CAC→AAC	rpoB3	75.5–76.0 (75.75)	72.3–72.4	3.4–3.5
His445Asn + *Asp435Glu	1	CAC→AAC; GAC→GAA	rpoB3; rpoB2	75.5–76.0 (75.75); 72.8–73.2 (73)	72.4; 70.2	3.4; 2.8
His445Tyr	4	CAC→TAC	rpoB3	75.5–76.0 (75.75)	72.5–72.6	3.2–3.3
*His445Arg	4	CAC→CGC	rpoB3	75.5–76.0 (75.75)	73.9	1.9
His445Arg + Ser428Arg	1	CAC→CGC; AGC→AGG	rpoB1	69.1–69.5 (69.3)	65.8	3.5
Ser450Phe	1	TCG→TTC	rpoB3	75.5–76.0 (75.75)	71.8	4.0
*Ser450Leu	14	TCG→TTG	rpoB3; rpoB4A	75.5–76.0 (75.75); 67.0–67.6 (67.3)	72.9–73.3; 73.3–73.8	2.5–2.9; 6.0–6.5
Ser450Leu + Thr482Asn	2	TCG→TTG; ACC→AAC	rpoB2; rpoB3; rpoB4A	72.8–73.2 (73); 75.5–76.0 (75.75); 67.0–67.6 (67.3)	69.2–69.5; 73.1–73.3; 73.6–73.7	3.5–3.8; 2.5–2.7; 6.3–6.4
Ser450Leu + Ile491Val	2	TCG→TTG; ATC→GTC	rpoB2; rpoB3; rpoB4A	72.8–73.2 (73); 75.5–76.0 (75.75); 67.0–67.6 (67.3)	70.0; 73.2–73.3; 73.6–73.7	3.0; 2.5–2.6; 6.3–6.4
*Ser450Trp	3	TCG→TGG	rpoB3; rpoB4A	75.5–76.0 (75.75); 67.0–67.6 (67.3)	73.1–73.5; 70.6–71.0	2.3–2.7; 3.3–3.7
Ser450Trp + *Ser431Gly	1	TCG→TGG; AGC→GGC	rpoB3; rpoB4A; rpoB1	75.5–76.0 (75.75); 67.0–67.6 (67.3); 69.1–69.5 (69.3)	73.2; 70.7; 66.4	2.6; 3.4; 2.9
**Leu452Pro*	12	CTG→CCG	rpoB4B	67.0–67.6 (67.3)	61.2–61.6	5.7–6.1
Ile491Phe	10	ATC→TTC	ND	ND	ND	ND

[a]Capturing probes, wild-type melt peak temperature (T_m) ranges and means, mutant T_m, ranges, and absolute values of melting temperature shift (ΔT_m) ranges associated with specific *rpoB* mutations in the strains tested and the corresponding nucleotide changes. ND, strains that harbored corresponding mutations outside the RRDR yielded a "RIF Resistance Not Detected" result. *, rifampin resistance-determining region (RRDR) mutation unambiguously identified by unique combinations of Ultra probes and ΔT_ms, including disputed ones (in italics). #, rifampin susceptible according to phenotypic testing.
[b]For double mutants, the high-confidence RR-conferring mutations are underlined (6, 7).

[a]Capturing probes, wild-type melt peak temperature (*Tm*) ranges and means, mutant *Tm* ranges, and absolute values of melting temperature shift (Δ*Tm*) ranges associated with specific *rpoB* mutations in the strains tested and the corresponding nucleotide changes. ND, strains that harbored corresponding mutations outside the RRDR yielded a "RIF Resistance Not Detected" result. *, rifampin resistance-determining region (RRDR) mutation unambiguously identified by unique combinations of Ultra probes and Δ*Tm*s, including disputed ones (in italics). #, rifampin susceptible according to phenotypic testing.

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SUPPLEMENTARY INFORMATION

Supplemental methods. Processing of rifampicin-resistant tuberculosis thermolysates for Xpert Ultra

testing: methodological details.

Figure S1. Interpreting Xpert Ultra ΔTm plus probe reactions observed in 2 rifampin-resistant tuberculosis patients for evaluation of a potential transmission event.

FigureS2. Interpreting Xpert Ultra ΔTm plus probe reactions observed in 2 rifampin-resistant tuberculosis patients for identification of relapse cases.

https://jcm.asm.org/highwire/filestream/123403/field_highwire_adjunct_files/0/zjm999096071s1.pdf