Evaluation of a Novel Line Probe Assay for the Timely Detection of Extensively Drug-Resistant Tuberculosis

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Revised abstract

Background: The novel Line Probe Assay (LPA) RDB-2185 TB Resistance (Genome Identification Diagnostics GmbH, Strassberg, Germany) is designed to indicate genetic events in *Mycobacterium tuberculosis* (Mtb) that are associated with resistance to isoniazid (H), rifampicin (R), streptomycin (S), kanamycin (K), amikacin (A) and fluoroquinolones (FQ) directly from smear-positive respiratory specimens. This technology enables the timely detection of multidrug-resistant Mtb (MDR TB) as well as extensively drug-resistant Mtb (XDR TB).

Methods: Drug susceptibilities of Mtb isolates obtained from Ukrainian patients with new pulmonary TB were assessed by the Bactec MGIT 960 method (Becton-Dickinson, Germany). The following drugs were tested at the critical concentrations (mg/L) indicated: H (0.1; 0.4), R (1.0), pyrazinamide (100), ethambutol (5.0; 7.5), S (1.0; 4.0), A (1.0), and lefofloxacin (L, 1.0). LPA RDB-2185 was performed in duplicate according to the manufacturer's instructions, in a blinded manner, from DNA obtained from the strains. Genotypic results were compared to the phenotype (reference).

Results: To date, 24 Mtb isolates have been evaluated. Of these, 15 (62.5%) were susceptible to all the drugs tested. Two (8.3%) showed mono-resistance to H, and three (12.5%) were resistant to both H and S. Three (12.5%) isolates were resistant to at least H and R, i.e. were MDR TB. One isolate (4.2%) was resistant to H, R, S, A, and L and therefore represents a XDR TB. There was a direct agreement for 21 of 24 Mtb isolates between standard drug susceptibility testing and the LPA RDB-2185. The turn-around-time of LPA RDB-2185 was approximately 4.5 hours.

Conclusions: The preliminary results indicate that LPA RDB-2185 may have a role to play in detecting drug-resistant Mtb in clinical specimens within a clinically much more relevant time frame than previously possible. Further evaluation of LPA RDB-2185 using smear-positive respiratory specimens and XDR TB strains is ongoing.

Background

Emergence of drug-resistant *Mycobacterium tuberculosis* (Mtb) strains is occurring throughout the world. Multidrug-resistant tuberculosis (MDR TB) is defined as resistance to both isoniazid (H) and rifampicin (R) which are considered the pillars of modern tuberculosis (TB) drug regimens (1). Extensively drug- resistant tuberculosis (XDR TB) is caused by a Mtb strain being resistant to at least H and R in addition to resistance to any fluoroquinolone and at least one of three injectable second line anti-tubercular drugs i.e. amikacin, kanamycin and/or capreomycin (2). Resistance to drugs in Mtb is chromosomally determined by missense mutations and/or deletions (3,4). The novel Line Probe Assay (LPA) RDB-2185 TB Resistance (Genome Identification Diagnostics GmbH, Strassberg, Germany) is designed to detect genetic events that are associated with resistance to isoniazid, rifampicin, streptomycin (S), kanamycin (K), amikacin (A) and fluoroquinolones (FQ) directly from smear-positive respiratory specimens (5). This technology potentially enables the timely detection of MDR TB as well as XDR TB.

Methods

Clinical Mtb strains (N = 24) from Ukrainian patients with new pulmonary TB were included in our study. (See abstract #0091148.)

LPA RDB-2185 was performed in duplicate according to the manufacturer's instructions, in a blinded manner, from DNA obtained from the strains.

Drug susceptibility testing (DST) was done by the Bactec MGIT 960 method (Becton-Dickinson, Germany). The critical concentrations in mg/L were: H (0.1; 0.4), R (1.0), pyrazinamide (100), ethambutol (5.0; 7.5), S (1.0; 4.0), A (1.0), and levofloxacin (L, 1.0). (See abstract #0091148.)

Genotypic and phenotypic results of the strains were compared by analyzing each set of antibiotics.

Results

Until now, 24 Mtb isolates have been tested (table 1 and figure 1). Of these,

- 15 (62.5%) were susceptible to all the drugs tested
- two (8.3%) showed mono-resistance to H
- three (12.5%) were resistant to both H and S
- three (12.5) isolates were resistant to at least H and R → MDR TB
- one (4.2%) isolate was resistant to H, R, S, A, and L → XDR TB
- 21 of 24 Mtb isolates directly agreed between direct standard drug susceptibility testing and LPA RDB-2185

The concordance of LPA RDB-2185 and DST for each single drug is shown in table 2.

Results, continued

Table 1. Summary of Genotypic and Phenotypic DST Results

N	H	R	S	K / A	FQ	Fig. 1	Phenotype
15	*inhA* + *katG* WT	*rpoB* WT	*rrs* + *rpsL* WT	*rrs* WT	*gyrA* WT	A	Susceptible
1	*inhA* + *katG* WT	*rpoB* WT	*rrs* + *rpsL* WT	*rrs* WT	*gyrA* WT		H > 0.4
1	MUT *katG*	*rpoB* WT	*rrs* + *rpsL* WT	*rrs* WT	*gyrA* WT	B	H > 0.4
1	MUT *inhA* + *katG*	*rpoB* WT	MUT *rrs*	*rrs* WT	*gyrA* WT		H > 0.4, S > 4.0
1	MUT *katG*	*rpoB* WT	MUT *rpsL*	*rrs* WT	*gyrA* WT		H > 0.4, S > 4.0
1	MUT *inhA*	*rpoB* WT	MUT *rpsL*	*rrs* WT	*gyrA* WT	C	H > 0.4, S > 4.0
1	MUT *katG*	MUT *rpoB* 516	*rrs* + *rpsL* WT	*rrs* WT	*gyrA* WT	D	H > 0.1< 0.4; R > 1.0, S > 1.0
1	MUT *katG*	MUT *rpoB* 531	*rrs* + *rpsL* WT	*rrs* WT	*gyrA* WT		H > 0.1< 0.4, R > 1.0
1	MUT *katG*	MUT *rpoB* 531	MUT *rpsL*	*rrs* WT	*gyrA* WT		H > 0.4, R > 1.0, S > 4.0
1	MUT *inhA* + *katG*	MUT *rpoB* 531	MUT *rpsL*	MUT *rrs*	*gyrA* WT	E	H > 0.4, R > 1.0 S > 4.0, A > 1.0, L >1.0

N = Number; H = Isoniazid; R = Rifampicin; S = Streptomycin; K / A = Kanamycin / Amikacin; FQ = Flouoroquinolone; WT = wild type; MUT = mutation

Figure 1. Patterns obtained by LPA RDB-2185 TB Resistance

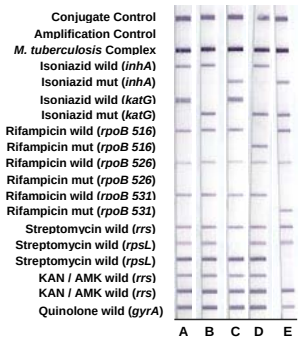

Table 2. Concordance of Genotypic and Phenotypic DST Results

Drug	N	Phenotype		Concordance
		missing (%)	resistant (%)	(%)
H	24	0	9 (37.5)	23 (95.8)
R	24	0	4 (16.7)	24 (100)
S	24	0	6 (25)	23 (95.8)
K / A	24	22 (91.7)	1 (42)	2 (100)
FQ	24	0	1 (42)	23 (95.8)

Conclusions

- Our results indicate that LPA RDB-2185 may have a role to play in the timely detection of drug-resistant Mtb in clinical specimens.
- In this population there is over 95% concordance of genotypic and phenotypic results regarding each single antibiotic.
- Since our data set is limited (N = 24) we have to analyze
  - a larger number of strains, including K / A and FQ resistant
  - smear-positive respiratory specimens
- The assay's performance will depend upon the nature of the genetic events leading to resistances in the Mtb strains encountered in a particular clinical setting.

References

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