Mutations Associated with Second-line Tuberculosis Drug Resistance in Georgia

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Introduction

- Georgia is a high-burdened MDR-TB Country
- WHO recommends rapid molecular tests (LPA & Xpert MTB/RIF) to diagnose TB & MDR-TB in high-burden countries
- Rapid Tests for XDR-TB are still limited & results have varied by geography
- Our prior study evaluating the MTBDR*sl* assay found less than optimal performance
  - OFL: SENS 81%, SPEC 99%
  - KAN: SENS 29%, SPEC 99%
  - CAP: SENS 57%, SPEC 94%

Study Aims

• To identify *gyrA* & *gyrB* genetic mutations associated with phenotypic Ofloxacin resistance

•To identify *rrs* & *eis* genetic mutations associated with phenotypic Kanamycin & Capreomycin resistance

•To evaluate if inclusion of additional mutations would improve performance of MTBDR*sl* in our setting

Methods

- **Study sites:**
  - National Center for TB and Lung Diseases, Tbilisi, Georgia
  - Public Health Research Institute (PHRI) TB Center in Newark, NJ
- **Study Design**
  - Retrospective study using stored M. *tuberculosis (MTB)* isolates (*from prior study on MTBDRsl performance*)
- **Laboratory**
  - Subculturing *MTB* isolates
  - DNA extraction (QIAamp DNA mini kit)
  - DNA sequencing: *gyr*A, *gyr*B, *rrs*, *eis* (Sanger sequencing, performed by Macrogen in NYC)
- **Data Analysis**
  - Performed with SAS v. 9.3 (Statistical Analysis Software Institute, Cary, NC)
  - Sensitivity/Specificity of MTBDR*sl* and DNA sequencing vs. DST

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Results

Performance parameters of *gyr*A/*gyr*B mutations in detecting any resistance to Ofloxacin, compared to conventional Drug Susceptibility Testing (2 µg/ml LJ), (reference standard) (n=111)

	gyrA	*gyrA + gyrB*
True Susceptible	95	95
True Resistant	12	14
False Susceptible	4	2
False Resistant	0	0
Sensitivity	**75**	**88**
Specificity	100	100
PPV	100	100
NPV	96	98

Performance parameters of *rrs*2/*eis*1 mutations in detecting any resistance to Capreomycin, compared to conventional Drug Susceptibility Testing (40 µg/ml LJ), (reference standard) (n=111)

	*rrs*2 (n=113)	*rrs*2+*eis*1 (n=111)
True Susceptible	93	70
True Resistance	8	10
False Susceptible	9	5
False Resistance	3	26
Sensitivity	47	67
Specificity	97	73
PPV	73	28
NPV	91	93

Performance parameters of *rrs*2/*eis*1 mutations in detecting any resistance to Kanamycin, compared to conventional Drug Susceptibility Testing (30 µg/ml LJ) (reference standard) (n=111)

	*rrs*2 (n=113)	*rrs*2+*eis*1 (n=111)	*rrs*2 + C-14T (n=111)
True Susceptible	49	43	49
True Resistance	11	31	17
False Susceptible	53	30	45
False Resistance	0	6	0
Sensitivity	**17**	51	**28**
Specificity	100	88	100
PPV	100	84	100
NPV	48	59	52

Mutations revealed in genes associated with the second line drug resistance by Gene Sequencing

gyrA	*gyrB*	*rrs2*	*eis1*
GCG (A)90GTG (V) GAC(D)94GGC(G)	G145A (CGT-CAT) C1628T (GCG-GTG)	A1401G	G-9C (CAG-CAC) G-10A (CAG-CAA) C-12T (CAG-TAG) C-14T (CCA-CTA) C159A (GGC-GGA)

Conclusions

- The inclusion of the *gyr*B gene may improve the sensitivity of the MTBDR*sl* assay for the detection of OFX resistance
- The inclusion of *eis* gene (C-14T), as a marker of Km resistance, would improve the sensitivity of rapid detection assays for Km resistance
- Additional *eis* mutations increased sensitivity for Km & Cm phenotypic resistance but have poor specificity
- In many MTB isolates Km and Cm resistance is not associated with known drug resistance mutations in *rrs* and *eis* genes; further work is needed to determine the mechanism of resistance in such cases