Analysis of *Mycobacterium tuberculosis* MDR/XDR Genes Using Next-Generation Ion Torrent Sequencing from Genetically Diverse South African Clinical Isolates

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INTRODUCTION

Mycobacterium tuberculosis (MTB), the causative agent for tuberculosis, is a highly transmissible bacterial pathogen with significant morbidity and mortality, particularly within an increasing number of reported multidrug resistant (MDR) and extensively drug resistant (XDR) strains.

MDR tuberculosis strains are resistant to first line antibiotics rifampin (RIF) and isoniazid (INH) while XDR MTB strains are resistant to both RIF and INH as well as fluoroquinolone and second-line injectable antibiotic drugs (e.g., amikacin, kanamycin or capreomycin).

Culture-based drug susceptibility testing (DST) of MDR strains is considered the gold-standard, but is time consuming (weeks to months), technically challenging and cost prohibitive. In recent years, commercially available nucleic acid based assays such as the GenoType MTBDR*plus* Line Probe Assay (LPA) by Hain LifeScience[1] have become widely utilized. Hain LPA will **NOT** detect amino acid mutations that are: 1) outside of known/characterized mutation sites, 2) from mixed-strain populations, and 3) within known resistance codons but result in new and previously uncharacterized amino acid substitutions.

SPECIFIC AIM

A relatively rapid (2-3 days), standardized, cost-effective protocol for full length gene analysis of *rpoB, katG, gyrA* and *rrs* MTB genes associated with first and second-line MTB drug resistance was developed and evaluated using the next-generation Ion Torrent Personal Genome Machine (PGM). MDR and XDR clinical Isolates (*N*=27) collected and transported in PrimeStore MTM[2] from South Africa to the US were compared to results obtained from the Hain LPA and culture.

METHODS

A panel of geographically diverse MDR and XDR clinical isolates (*N*=27) from South Africa were collected and shipped at ambient temperature in PrimeStore MTM. Four full-length TB genes: *rpoB* (rifampin), *katG* (isoniazid), *gyrA* (ofloxicin and fluorochinolone) and *rrs* (amikacin, kanamycin and capreomycin) were PCR amplified, sequenced with the Ion Torrent PGM and genetically characterized using LaserGene DNAStar software (Figure 1). Ion Torrent sequences were compared to HAIN Line Probe Assay (LPA) and DST using the BACTEC™ MGIT™ 960.

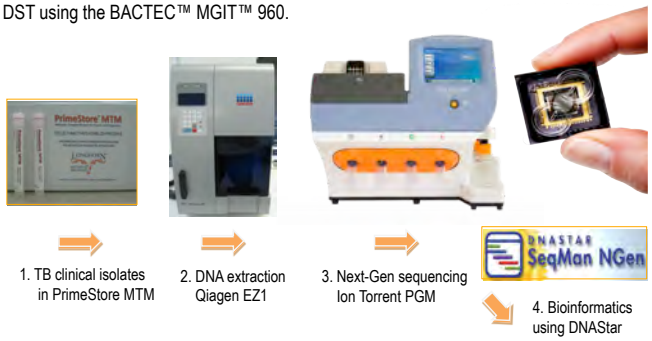

Figure 1. Methodology for MTB drug resistance sequencing on the Ion Torrent PGM.

RESULTS

Polymerase chain reaction (PCR) was performed using 4 sets of novel primer pairs for full length amplification of *rpoB, katG, gyrA* and *rrs* genes associated with MDR and XDR resistance in MTB (Figure 2).

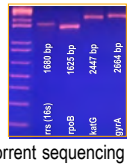

Figure 2. Representative amplicons for 4 MTB genes (*rpoB, katG, gyrA*, and *rrs*). A total of 8,416 bps for four genes was sequenced for each clinical isolate. A 1.0% agarose gel with 1 KB ladder is shown.

Compared to Hain Line Probe Assay (LPA) and culture DST, the Ion Torrent sequencing method correctly detected amino acid substitutions from 27 of 27 multiplexed MTB strains representing genetically diverse MDR and XDR antibiotic resistance patterns (Table 1). Furthermore, several uncommon amino acid mutations were discovered outside of known drug resistance codons that were not noted by LPA analysis. Table 2 reveals amino acid substitutions in the *rpoB* gene using Ion Torrent sequencing compared to Hain LPA and culture (Table 2).

RESULTS

Table 1. Comparison of Drug Resistance in *M. tuberculosis* South African clinical isolates according to Ion Torrent genotype, HAIN LPA and culture DST.

No. of Strains (*N*=27)	RIF	INH	FQ	2nd Line Abs
Ion Torrent	21 (78%)	16 (59%)	10 (37%)	7(26%)
Hain LPA	20* (74%)	16 (59%)	N/A	N/A
Culture DST	21 (78%)	16 (59%)	10 (37%)	7(26%)

*RIF = Rafampin, INH = Isoniazid, FQ = Fluoroquinolone, 2nd Line Abs = Kanamycin, Amikacin, Capreomycin

*One clinical isolate was inconclusive by Hain LPA

Isolate Designation	*rpoB* gene: rpoB mutation (amino acid position) Ion Torrent PGM						Antibiotic Profile (Sensitivity/Resistance)		
	194	509	516*	526*	531*	533	Ion Torrent PGM genotype	Hain GenoType® MTBDRplus	BACTEC™ MGIT™ 960
H37Rv reference	V	S	D	H	S	L	Sensitive	Sensitive	Sensitive
2919984	V	S	D	H	L	L	Resistant	Resistant	Resistant
2961678	V	S	D	H	L	L	Resistant	Resistant	Resistant
3050732	V	S	D	D	S	L	Resistant	Resistant	Resistant
2875244	V	R	D	Y	S	L	Resistant	Resistant	Resistant
2937843	V	S	D	H	L	L	Resistant	Resistant	Resistant
2997164	V	S	D	H	L	L	Resistant	Resistant	Resistant
Strain 001	V	S	D	H	S	L	Sensitive	Sensitive	Sensitive
Strain 003	V	S	D	H	S	L	Sensitive	Sensitive	Sensitive
Strain 005	V	S	D	H	S	L	Sensitive	Sensitive	Sensitive
Strain 006	V	S	D	H	S	L	Sensitive	Sensitive	Sensitive
Strain 007	V	S	D	H	S	L	Sensitive	Sensitive	Sensitive
NS1	V	S	D	H	S	L	Sensitive	Sensitive	Sensitive
14641	V	S	G	H	S	P	Resistant	Resistant	Resistant
14655	V	S	G	H	S	P	Resistant	Resistant	Resistant
14666	V	S	G	H	S	P	Resistant	Resistant	Resistant
14669	V	S	D	H	L	L	Resistant	Resistant	Resistant
14671	V	S	G	H	S	P	Resistant	Resistant	Resistant
14949	V	S	G	H	S	P	Resistant	Resistant	Resistant
14965	V	S	G	H	S	P	Resistant	Resistant	Resistant
3290183	I	S	D	H	L	L	Resistant	Resistant	Resistant
3174991	V	S	D	H	L	L	Resistant	Resistant	Resistant
3247469	V	S	D	H	L	L	Resistant	Resistant	Resistant
3275726	V	S	D	H	L	L	Resistant	Resistant	Resistant
3150565	V	S	D	R	S	L	Resistant	Inconclusive‡	Resistant
3100029	V	S	D	H	L	L	Resistant	Resistant	Resistant
3264812	V	S	D	H	L	L	Resistant	Resistant	Resistant
14657	V	S	V	H	S	L	Resistant	Resistant	Resistant

‡An uncommon Arginine (Arg) amino acid substitution at position 526 was not detected by HAIN LPA

Table 2. Characterization of amino acid changes in *the rpoB* gene from 27 clinical isolates obtained from South Africa using Ion Torrent PGM sequencing. Ion Torrent genotype results in known resistance codons were compared to Hain GenoType MTBDRplus and BACTEC MGIT 960 culture results. An inconclusive result by HAIN was shown to be an uncommon amino acid substitution (H526R) by Ion Torrent sequencing.

CONCLUSIONS

➢The developed Ion Torrent method characterizes MDR and XDR strains with overall performance comparable to Hain LPA testing and offers potential discovery of novel resistance mutations.

➢This developed gene chip will facilitate tracking and monitoring of geographically diverse MDR/XDR strains and discovery of new TB resistance mutations.

➢Ion Torrent sequencing allows for increased scale-up to include additional TB genes.

➢Our developed protocol does not use expensive ancillary equipment, requires a relatively small working footprint, is considerably more cost efficient compared to other next-generation platforms and can be potentially integrated into resource limited environments.

REFERENCES

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