Published in final edited form as:

Int J Tuberc Lung Dis. 2016 February ; 20(2): 160–165. doi:10.5588/ijtld.15.0459.

Performance of a Pyrosequencing Platform in Diagnosing Drug-resistant Extrapulmonary Tuberculosis Specimens in India

Sophia B Georghiou^a, Kanchan Ajbani^b, Camilla Rodrigues^b, and Timothy C Rodwell^a

^aDivision of Global Public Health, University of California, San Diego, School of Medicine, La Jolla, California, United States of America

^bSection Microbiology, Department of Laboratory Medicine, P.D. Hinduja National Hospital and Medical Research Center, Mumbai, India

Abstract

SETTING—Pyrosequencing diagnostic assays have shown great utility in identifying and characterizing pulmonary drug-resistant tuberculosis (DR-TB) infections. However, the method has yet to be evaluated for the diagnosis of drug-resistant extrapulmonary tuberculosis (DR-EPTB) infections.

OBJECTIVE—To evaluate the performance of a pyrosequencing platform in establishing molecular drug-resistance profiles for 79 clinical EPTB specimens at a DR-TB referral center in India.

DESIGN—Genotypic drug-resistance profiles were established for all 79 non-pulmonary, culture-positive tuberculosis clinical specimens. Acid-fast bacilli smears, MGIT960 cultures and drug-susceptibility testing were performed on all specimens for reference.

RESULTS—In comparison to MGIT960, the sensitivity and specificity of pyrosequencing in detecting drug-resistance among the specimens was found to be 100% and 100%, 67% and 98%, and 100% and 100% for isoniazid, rifampicin, and the fluoroquinolones, respectively. No EPTB specimens were phenotypically resistant to any of the injectables, but specificity of the assay was determined to be 100%, 98%, and 98% for amikacin, kanamycin, and capreomycin.

CONCLUSIONS—Pyrosequencing is a rapid, appropriate technology for the diagnosis of isoniazid-, fluoroquinolone-, and potentially injectable-resistant EPTB clinical specimens and should be considered as an alternative to conventional growth-based diagnostic methods for EPTB when resistance to these drugs is suspected.

Keywords

molecular diagnostics; genetic mutations; drug resistance

Corresponding author: Sophia Georghiou, Stein Clinical Research Building, University of California, San Diego, 9500 Gilman Drive, La Jolla, California 92093, Tel. 1-805-975-6349, sgeorghi@ucsd.edu.

AUTHOR CONTRIBUTIONS

SG executed study, performed analyses, interpreted results and wrote manuscript; KA executed study, reviewed manuscript; CR supervised and advised study, reviewed manuscript; TR designed and advised study, reviewed manuscript.

INTRODUCTION

Tuberculosis (TB) is one of the world's deadliest infectious diseases. In 2013, 9 million people contracted TB and 1.5 million died of the disease.¹ India has the highest number of incident TB cases in the world, representing 40% of the global TB burden.¹ The bacterium that causes TB, *Mycobacterium tuberculosis* (*Mtb*), most commonly infects the lungs, although any organ or tissue can be involved. Extrapulmonary TB (EPTB) infections account for over 18% of all TB cases reported in India.¹ EPTB clinical presentation is atypical, so diagnosis relies on strong clinical suspicion and laboratory confirmation of infection. Unfortunately, conventional laboratory methods for diagnosing EPTB are based on slow culture of *Mtb* isolated from infected organs, resulting in treatment delays and a high EPTB case fatality rate. In India, EPTB infections have a 25–50% mortality rate.² Additionally, from 2012 to 2013, the number of reported drug-resistant TB (DR-TB) cases in India increased by 65%. Timely diagnosis and initiation of chemotherapy is key to the successful treatment of DR-EPTB patients.³ However, conventional culture and drug susceptibility testing (DST) methods can take months to complete.

Molecular assays such as pyrosequencing, based on the detection of resistance-conferring mutations in *Mtb*, are promising methods for rapid DR-TB diagnosis as they are not limited by slow culture methods. To our knowledge, only one laboratory study to date has examined the utility of pyrosequencing for a small set of non-pulmonary TB samples.⁴ While the technology is effective for rapid detection of DR-TB in pulmonary samples,^{5–7} it is also necessary to assess its performance for non-pulmonary samples to support its use for clinical detection of DR-EPTB. The objective of this study was to evaluate the performance of a pyrosequencing platform in establishing molecular drug-resistance profiles of 79 extrapulmonary clinical specimens at a DR-TB referral center in India.

METHODS

Clinical Site

All experiments were performed at the Mycobacteriology Laboratory of P. D. Hinduja National Hospital and Medical Research Center in Mumbai, India.

Ethical Approval

This study was approved by the IRBs of Hinduja Hospital and the University of California, San Diego. Written consent was waived for all participants as the study was carried out on archived clinical sediments. Pyrosequencing to Diagnosis Extrapulmonary TB

Specimens

Seventy-nine clinical specimens were used for this study. Specimens were collected from consecutive patients suspected of having EPTB that visited Hinduja Hospital during routine care visits in 2014. Clinical samples were of diverse origin: pus, abscess and cold abscess (n= 31), tissue and biopsies (n= 28), lymph node (n= 17), cerebrospinal fluid (n= 1), and other types (pleural fluid or fine needle aspiration cytology) (n= 2). At time of collection all specimens were subjected to acid-fast bacilli (AFB) smear, LJ and MGIT960 culture

(Becton Dickinson Biosciences, Sparks, MD, USA). Following collection, all specimens were stored as sediments at -80°C until DNA could be extracted for pyrosequencing. For this study, samples were revived in liquid media and DST was performed via MGIT960. Only TB culture positive specimens with valid DST results were used to ensure that each sample had a clear reference standard diagnosis.

Phenotypic Resistance Profiles

Phenotypic DST was performed using MGIT960 with EpiCenter software (Becton Dickinson, Franklin Lakes, NJ, USA) following standard manufacturer protocols.⁸ All clinical specimens were tested for phenotypic resistance to isoniazid (INH), rifampicin (RIF), moxifloxacin (MOX), ofloxacin (OFX), amikacin (AMK), kanamycin (KAN), and capreomycin (CAP). WHO-recommended critical concentrations were used to determine phenotypic resistance to each drug.⁹ The one exception was KAN, which was tested at $2.5\mu\text{g/mL}$ in accordance with published literature.¹⁰ DST was repeated once for isolates with conflicting molecular test results for INH or RIF.

Genotypic Resistance Profiles

Nucleic acids were extracted from all sediments as previously published.⁴ PCR was performed for each sample, and pyrosequencing reactions were conducted using a repurposed Qiagen PyroMark Q96 ID system (Qiagen, Valencia, CA, USA)^{4, 7} In brief, the pyrosequencing assay included eight subassays performed in parallel. *Mtb* presence was confirmed by detection of the *IS6110* marker; INH resistance by mutations in *katG* codons 312–316, the *inhA* promoter position -4 to -20 and the *ahpC* promoter position -4 to -23 ; RIF resistance by mutations in *rpoB* codons 507–533; fluoroquinolone (MOX and OFX) resistance by mutations in *gyrA* codons 88–95; and injectable (AMK, KAN and CAP) resistance by mutations in the 1401 region of the *rrs* gene. For INH resistance determination, any specimen containing a resistance associated mutation in *katG*, the *inhA* promoter or *ahpC* promoter was considered genotypically INH-resistant. However, for a specimen to be genotypically INH-susceptible, all three gene regions had to be genetically wildtype. For RIF resistance determination, any specimen with a resistance-associated mutation in the RRDR region of *rpoB* was considered RIF-resistant. However, both *rpoB* regions evaluated (codons 507–521 and codons 522–533) had to be wildtype for the specimen to be called RIF-susceptible. Fluoroquinolone and injectable genotypic resistance profiles were determined solely based upon the presence or absence of resistance-associated mutations in the respective *gyrA* and *rrs* gene regions sequenced. Sequences were confirmed automatically following pyrosequencing by IdentiFire (Qiagen, Valencia, CA, USA) software analysis. All gene targets were pyrosequenced once for every isolate, and those targets that did not obtain 100% sequence alignments with IdentiFire library sequences were repeated once, increasing the final concentration of the respective sequencing primer from $0.4\mu\text{M}$ to $0.6\mu\text{M}$. If this second reaction also did not return a 100% sequence alignment, results were deemed indeterminate. Based upon generated sequencing reads, specimens were classified as genotypically resistant, susceptible, or indeterminate for each drug.

Data Analysis

AFB smear results were plotted by specimen type, and time to culture positivity was noted for all specimens. Pyrosequencing platform performance was determined by comparing PyroMark genotypic findings to conventional MGIT960 DST results. Sensitivity and specificity of the assay was calculated for each drug of interest. Frequencies of mutations were also calculated among the phenotypically resistant and susceptible specimens in the study for which gene sequences were obtained.

RESULTS

Specimens

Only 17% of specimens were positive for *Mtb* by AFB smear: 1 pus sample was occasional, 3 pus samples were positive 1+, and 9 pus, biopsy, lymph node, and tissue samples were occasional 2 bacilli. 82% of specimens were smear negative. One biopsy sample did not have sufficient material to perform a smear. Specimen smear status by sample type is reported in Chart 1. Time to culture positivity (data not shown) ranged from 15 days to 86 days. Average time to culture positivity was 34 days with a standard deviation of 15 days.

Phenotypic Resistance Profiles

MGIT960 DST found 13.9% (11/79) of specimens to be multidrug-resistant TB-resistant to both INH and RIF. Of these, 10.1% (8/79) had additional resistance to at least one of the fluoroquinolones. 17.7% (14/79) of specimens were mono-resistant to INH, and 3.8% (3/79) of specimens were mono-resistant to RIF. One specimen was resistant only to the fluoroquinolones. No extremely drug-resistant TB specimens, or multidrug-resistant TB with additional resistance to a fluoroquinolone and at least one injectable, were identified in this study. 63.3% (50/79) of specimens were susceptible to all drugs tested.

Pyrosequencing Diagnostic Performance

***Mtb* detection**—Of the 79 specimens evaluated, 73 were confirmed to contain *Mtb* via identification of the IS6110 marker.

INH-resistance—Pyrosequencing demonstrated 100% sensitivity, specificity, and agreement for the detection of INH resistance in comparison to phenotypic DST (Table 1). Twenty-two (37.9%) were phenotypically resistant to INH, as all contained resistance-associated mutations in *katG* or the *inhA* promoter (Table 2). Thirty-six specimens were phenotypically susceptible to INH, as none had a resistance-associated mutation in any of the three genes evaluated. Twenty-one specimens did not return a sequencing read for at least one of these three genes, and did not identify any resistance-associated mutations, making them genotypically indeterminate for INH resistance.

RIF-resistance—For detection of RIF resistance, test sensitivity was determined to be 67%, while specificity was 98% and agreement between the phenotypic and genotypic tests was 93% (Table 1). Twelve specimens were phenotypically resistant to RIF. Of these, only eight contained resistance-associated mutations in the *rpoB* gene regions evaluated (Table 2). Only one of 55 RIF-susceptible specimens contained an *rpoB* resistance-associated

mutation. Twelve samples were genotypically indeterminate for RIF resistance-not returning a sequencing read for at least one of the two *rpoB* gene regions sequenced.

Fluoroquinolone resistance—Based upon availability of pyrosequencing data for the *gyrA* gene region, 47 specimens were evaluated for MOX- and OFX-resistance. Pyrosequencing demonstrated 100% sensitivity, specificity and agreement for the detection of phenotypic fluoroquinolone resistance (Table 1). Of the seven fluoroquinolone-resistant samples, all seven had resistance-associated mutations in *gyrA* (Table 2). Forty specimens were phenotypically susceptible to both MOX and OFX, none containing resistance-associated mutations in *gyrA*, though all contained the 95ACC mutation, associated with fluoroquinolone susceptibility.¹¹

Injectable resistance—Although no injectable-resistant isolates were included in this study, the 1401 region of the *rrs* gene yielded sequencing data for 66 injectable-susceptible specimens. Test specificity was 100% for AMK but 98% for KAN and CAP (Table 1), as one isolate was found to have a mutation associated with KAN and CAP resistance (Table 2).

DISCUSSION

Pyrosequencing was shown to be a suitable rapid molecular diagnostic for the identification and characterization of *Mtb* drug resistance profiles from clinical EPTB samples. Full or partial sequence data was obtained for 79 diverse, culture positive EPTB specimens. Sensitivity and/or specificity was near perfect (~100%) for INH, the fluoroquinolones, and the injectables. Furthermore, molecular results were obtained within 1–2 days after sample processing, as opposed to the average 34 days needed to determine culture positivity and drug resistance profiles using conventional phenotypic methods.

The *Mtb* marker, IS6110, was identified in 92.4% (73/79) of EPTB specimens in our study. Although the marker was not identified in six specimens, the DR-TB primers used in our pyrosequencing platform were designed to be highly specific for the *Mycobacterium* TB complex,⁴ and so the presence of *Mtb* was confirmed as long as sequence data was obtained for one other targeted gene region. Additionally, there are TB strains missing the IS6110 marker in regions of Southern India.^{12, 13} For these reasons, the lack of the IS6110 marker among a low proportion of our specimens was not determined to compromise the technical performance of the assay. However, future versions of the assay would benefit from adding a second confirmatory marker, such as MPB-64, to confirm *Mtb* presence when IS6110 is absent.^{14, 15}

The pyrosequencing assay was able to detect a wide range of resistance-associated mutations in clinical EPTB specimens. The 100% sensitivity of pyrosequencing for the detection of INH, MOX, and OFX resistance suggests that the respective gene regions evaluated were sufficient to predict phenotypic drug resistance in our sample. Additionally, the assay had 100% specificity for these compounds, since it did not detect resistance-associated mutations in the respective gene regions of the drug susceptible specimens evaluated. Therefore, the assay is appropriate for determination of INH- and

fluoroquinolone-resistance profiles in EPTB specimens. As fluoroquinolones are increasingly being used to treat DR-TB infections, the high performance of the assay in detecting drug resistance to this particular drug class is clinically significant.

The sensitivity and specificity of pyrosequencing for the detection of RIF resistance was 67% and 98%, respectively. The assay did not detect resistance-associated mutations in four of the 12 phenotypically RIF-resistant specimens included in this study. These four discrepant specimens were sequence-confirmed by the MTBDR*plus* assay (Hain Lifescience, Germany), which is designed to detect most of the same *rpoB* mutations as pyrosequencing. DST was also repeated for these isolates, and the specimens were confirmed to be phenotypically RIF-resistant, indicating that these phenotypically RIF-resistant strains appeared not to have the expected *rpoB* mutations found in over 95% of RIF-resistant strains, globally.¹⁶ This discrepancy is likely due to the presence of mixed populations of *Mtb* in the EPTB samples at levels too low to be detected by the molecular tests, though they could be selected for by phenotypic DST methods, resulting in resistant phenotypes.¹⁷ This finding could also be an artifact of the small sample size of this study, as the confidence intervals for specificity included values as high as 88%. However, it might also be worth investigating *rpoB* gene regions outside of the regions evaluated by the molecular tests, to ensure that no rare mutations were present in these samples. Additionally, one RIF-susceptible specimen had a 522TTG mutation. This mutation has been previously characterized as conferring low levels of RIF-resistance (MICs 16–32ug/mL).^{18, 19} However, this mutation is rare, and RIF MICs for mutation have only been established in two known studies. The mutation should be further investigated to confirm its role in RIF resistance.

Although no injectable-resistant specimens were included in this study, 66 specimens provided sequence data for the *rrs* gene. Only one of these AMK-, KAN- and CAP-susceptible isolates was found to have a CAP and KAN resistance-associated mutation (C1402T), giving the assay a specificity of 98% for the detection of CAP and KAN resistance, and 100% for the detection of AMK resistance. The *rrs* 1402T mutation has been previously associated with both KAN and CAP resistance,^{20, 21} yet follow-up studies have shown this mutation to only convey resistance to CAP,^{22, 23} suggesting that its role in KAN resistance should be further evaluated. The finding of this resistance-associated mutation in one CAP-susceptible isolate may be explained by the WHO-recommended critical concentration for MGIT960 used in this study, since it has been demonstrated that this critical concentration is substantially higher than the epidemiological cutoff separating wildtype *Mtb* from those with CAP resistance-associated mutations.^{24, 25}

One limitation of this study was the failure of the pyrosequencing assay to make drug susceptibility calls for many of the *Mtb* targets in the EPTB specimens. Sequencing failures appeared to be associated with the smear status of the sample (smear negative samples had more sequencing failures) and the sequencing length of the given gene target (the longer *gyrA* and *ahpC* sequencing targets had more sequencing failures). As such, the performance of pyrosequencing appears to depend on the bacterial load of the sample as well as the length of the sequencing target. Although all targets were not sequenced for every isolate, sequencing hits were seen for every sample and every target, suggesting that pyrosequencing

has the potential to perform even for low DNA-yield samples and difficult targets. As such, the assay has the potential for increased sequencing success upon assay optimization.

In conclusion, our pyrosequencing assay shows excellent sensitivity and specificity for the prediction of phenotypic resistance to INH and the fluoroquinolones in culture positive EPTB clinical specimens. Additionally, pyrosequencing was shown to be a useful method for confirming injectable susceptibility, and may be potentially used to determine injectable resistance. The performance of the assay in regards to detection of RIF resistance should be further evaluated. Our results are significant in that they find pyrosequencing to be a rapid, alternative method to diagnose DR-EPTB while waiting for phenotypic confirmation of drug susceptibility by slower culture methods. Ultimately, pyrosequencing-based diagnostics provide clinically relevant *Mtb* sequence data, allowing treatment providers to rapidly diagnose EPTB from non-pulmonary clinical samples and make timely treatment decisions, even in the absence of smear results.

Acknowledgments

We wish to thank Dr. Antonino Catanzaro for providing advice on the study design. Funding for this study was provided by Hinduja Hospital and Medical Research Center. TCR and SG were supported by funding from the Global Consortium for Drug-resistant TB Diagnostics (GCDD; <http://gcdd.ucsd.edu>) which is supported by the National Institute of Allergy and Infectious Diseases (NIAID) grant U01-AI082229. TCR was also supported by NIAID grant R01-AI11435.

References

1. WHO. Global Tuberculosis Report 2014. Geneva, Switzerland: WHO; 2014.
2. Vadwai V, Boehme C, Nabeta P, Shetty A, Alland D, Rodrigues C. Xpert MTB/RIF: a new pillar in diagnosis of extrapulmonary tuberculosis? *Journal of clinical microbiology*. 2011; 49(7):2540–5. [PubMed: 21593262]
3. Jawahar MS, Rajaram K, Sivasubramanian S, et al. Treatment of lymph node tuberculosis--a randomized clinical trial of two 6-month regimens. *Tropical medicine & international health : TM & IH*. 2005; 10(11):1090–8. [PubMed: 16262733]
4. Lin SY, Rodwell TC, Victor TC, et al. Pyrosequencing for rapid detection of extensively drug-resistant *Mycobacterium tuberculosis* in clinical isolates and clinical specimens. *Journal of clinical microbiology*. 2014; 52(2):475–82. [PubMed: 24478476]
5. Bravo LT, Tuohy MJ, Ang C, et al. Pyrosequencing for rapid detection of *Mycobacterium tuberculosis* resistance to rifampin, isoniazid, and fluoroquinolones. *Journal of clinical microbiology*. 2009; 47(12):3985–90. [PubMed: 19846642]
6. Engstrom A, Morcillo N, Imperiale B, Hoffner SE, Jureen P. Detection of first- and second-line drug resistance in *Mycobacterium tuberculosis* clinical isolates by pyrosequencing. *Journal of clinical microbiology*. 2012; 50(6):2026–33. [PubMed: 22461677]
7. Ajbani K, Lin SY, Rodrigues C, et al. Evaluation of pyrosequencing for detecting extensively drug-resistant *Mycobacterium tuberculosis* among clinical isolates from four high-burden countries. *Antimicrobial agents and chemotherapy*. 2015; 59(1):414–20. [PubMed: 25367911]
8. Diagnostics, F. Section II: Procedure for Primary Isolation (Preparation and Inoculation for Culture: Detection of positive growth): FIND. 2006. MGIT Procedure Manual.
9. WHO. Updated interim critical concentrations for first-line and second-line DST (as of May 2012). 2012. http://www.stoptb.org/wg/gli/assets/documents/Updatedcriticalconcentrationtable_1stand2ndlinedrugs.pdf
10. Rodrigues C, Jani J, Shenai S, Thakkar P, Siddiqi S, Mehta A. Drug susceptibility testing of *Mycobacterium tuberculosis* against second-line drugs using the Bactec MGIT 960 System. *The*

international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease. 2008; 12(12):1449–55.

11. Lau RW, Ho PL, Kao RY, et al. Molecular characterization of fluoroquinolone resistance in *Mycobacterium tuberculosis*: functional analysis of *gyrA* mutation at position 74. *Antimicrobial agents and chemotherapy*. 2011; 55(2):608–14. [PubMed: 20956608]
12. Das S, Paramasivan CN, Lowrie DB, Prabhakar R, Narayanan PR. IS6110 restriction fragment length polymorphism typing of clinical isolates of *Mycobacterium tuberculosis* from patients with pulmonary tuberculosis in Madras, south India. *Tubercle and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease*. 1995; 76(6):550–4.
13. Narayanan S, Parandaman V, Narayanan PR, et al. Evaluation of PCR using TRC(4) and IS6110 primers in detection of tuberculous meningitis. *Journal of clinical microbiology*. 2001; 39(5): 2006–8. [PubMed: 11326036]
14. Sharma K, Sharma A, Sharma SK, Sen RK, Dhillon MS, Sharma M. Does multiplex polymerase chain reaction increase the diagnostic percentage in osteoarticular tuberculosis? A prospective evaluation of 80 cases. *International orthopaedics*. 2012; 36(2):255–9. [PubMed: 21442201]
15. Mehta PK, Raj A, Singh N, Khuller GK. Diagnosis of extrapulmonary tuberculosis by PCR. *FEMS immunology and medical microbiology*. 2012; 66(1):20–36. [PubMed: 22574812]
16. Lacombe A, Garcia-Sierra N, Prat C, et al. GenoType MTBDRplus assay for molecular detection of rifampin and isoniazid resistance in *Mycobacterium tuberculosis* strains and clinical samples. *Journal of clinical microbiology*. 2008; 46(11):3660–7. [PubMed: 18784319]
17. Colman RE, Schupp JM, Hicks ND, et al. Detection of Low-Level Mixed-Population Drug Resistance in *Mycobacterium tuberculosis* Using High Fidelity Amplicon Sequencing. *PloS one*. 2015; 10(5):e0126626. [PubMed: 25970423]
18. Williams DL, Spring L, Collins L, et al. Contribution of *rpoB* mutations to development of rifamycin cross-resistance in *Mycobacterium tuberculosis*. *Antimicrobial agents and chemotherapy*. 1998; 42(7):1853–7. [PubMed: 9661035]
19. Moghazeh SL, Pan X, Arain T, Stover CK, Musser JM, Kreiswirth BN. Comparative antimycobacterial activities of rifampin, rifapentine, and KRM-1648 against a collection of rifampin-resistant *Mycobacterium tuberculosis* isolates with known *rpoB* mutations. *Antimicrobial agents and chemotherapy*. 1996; 40(11):2655–7. [PubMed: 8913484]
20. Georghiou SB, Magana M, Garfein RS, Catanzaro DG, Catanzaro A, Rodwell TC. Evaluation of genetic mutations associated with *Mycobacterium tuberculosis* resistance to amikacin, kanamycin and capreomycin: a systematic review. *PloS one*. 2012; 7(3):e33275. [PubMed: 22479378]
21. Maus CE, Plikaytis BB, Shinnick TM. Molecular analysis of cross-resistance to capreomycin, kanamycin, amikacin, and viomycin in *Mycobacterium tuberculosis*. *Antimicrobial agents and chemotherapy*. 2005; 49(8):3192–7. [PubMed: 16048924]
22. Jugheli L, Bzekalava N, de Rijk P, Fissette K, Portaels F, Rigouts L. High level of cross-resistance between kanamycin, amikacin, and capreomycin among *Mycobacterium tuberculosis* isolates from Georgia and a close relation with mutations in the *rrs* gene. *Antimicrobial agents and chemotherapy*. 2009; 53(12):5064–8. [PubMed: 19752274]
23. Suzuki Y, Katsukawa C, Tamaru A, et al. Detection of kanamycin-resistant *Mycobacterium tuberculosis* by identifying mutations in the 16S rRNA gene. *Journal of clinical microbiology*. 1998; 36(5):1220–5. [PubMed: 9574680]
24. Angeby K, Jureen P, Kahlmeter G, Hoffner SE, Schon T. Challenging a dogma: antimicrobial susceptibility testing breakpoints for *Mycobacterium tuberculosis*. *Bulletin of the World Health Organization*. 2012; 90(9):693–8. [PubMed: 22984314]
25. Jureen P, Angeby K, Sturegard E, et al. Wild-type MIC distributions for aminoglycoside and cyclic polypeptide antibiotics used for treatment of *Mycobacterium tuberculosis* infections. *Journal of clinical microbiology*. 2010; 48(5):1853–8. [PubMed: 20237102]

Chart 1.
Characteristics of the 79 Extrapulmonary Tuberculosis Specimens included in Study.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Diagnostic Performance of Pyrosequencing for the Prediction of Phenotypic Drug-resistance among Extrapulmonary Tuberculosis Specimens (n=79).

Table 1

	INH (n=58)	RIF (n=67)	MOX (n=47)	OFX (n=47)	AMK (n=66)	KAN (n=66)	CAP (n=66)
Sensitivity	100%	67%	100%	100%	N/A	N/A	N/A
Specificity	100%	98%	100%	100%	100%	98%	98%
Agreement	100%	93%	100%	100%	100%	98%	98%
INH= Isoniazid							
RIF= Rifampicin							
MOX= moxifloxacin							
OFX= ofloxacin							
AMK= amikacin							
KAN= kanamycin							
CAP= capreomycin							

Table 2
Frequency of Mutations Identified among the Extrapulmonary Tuberculosis Specimens Evaluated in this Study (n=79).

GENE	MUTATION	DRUG	# DST R	# DST S	# PSQ R	# PSQ S	Frequency in R	Frequency in S
<i>katG</i>	315ACC	INH	22	36	20	0	91%	0%
	315ACA	INH	22	36	1	0	5%	0%
<i>inhA</i> -promoter	-15T	INH	22	36	1	0	5%	0%
	531TTG	RIF	12	55	5	0	42%	0%
<i>rpoB</i>	511CCG	RIF	12	55	1	0	8%	0%
	511CCG & 516GGC	RIF	12	55	1	0	8%	0%
	531TGG	RIF	12	55	1	0	8%	0%
	522TTG	RIF	12	55	0	1	0%	2%
	94GGC & 95ACC	MOX	7	40	3	0	43%	0%
<i>gyrA</i>	90GTG	MOX	7	40	1	0	14%	0%
	90GTG & 95ACC	MOX	7	40	1	0	14%	0%
	94GCC & 95ACC	MOX	7	40	1	0	14%	0%
	94AAC & 95ACC	MOX	7	40	1	0	14%	0%
	95ACC	MOX	7	40	0	38	0%	95%
<i>rrs</i>	1402T	AMK	0	66	0	1	N/A	2%
		KAN	0	66	1	0	N/A	0%
		CAP	0	66	1	0	N/A	0%

INH= Isoniazid

RIF= Rifampicin

MOX= Moxifloxacin

OFX= Ofloxacin

AMK= Amikacin

KAN= Kanamycin
CAP= Capreomycin
R= Resistant
S= Susceptible

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript