Tuberculosis Drug Resistance and Outcomes among Tuberculosis Inpatients in Lilongwe, Malawi

Charles Vorkas[1], Dumbani Kayira[2],Charles van der Horst[1], Irving Hoffman[1], Mina Hosseinipour[1,2] Creto Kanyemba[2], Nelson Nguluwe, Tarsizio Chikaonda[2], Maximina Kalikhoka[2], Doreen Kalaundi[2], Dan Namarika[2], Peter Gilligan[5] and Robert Krysiak[2]

1.University of North Carolina Hospitals Department of Medicine
2.UNC project, Lilongwe, Malawi.

Abstract

Setting/Objective: We evaluated clinical characteristics, yield of solid vs. liquid culture, polymerase chain reaction (PCR)-based drug-resistance profiles, and clinical outcomes of tuberculosis (TB) inpatients in Lilongwe, Malawi.

Design: We enrolled adult patients admitted to the Bwaila TB Ward from Jan-Aug/2010. Evaluations included questionnaires, clinical exam, chest radiograph, HIV status, CD4 lymphocyte count, plasma HIVRNA and sputum analysis including Auramine-O stain, Lowenstein-Jensen (LJ) and Mycobacterial Growth Indicator Tube (MGIT) culture, and susceptibility testing using the HAIN GenoType® MTBDRplus.

Results: Eighty-eight patients were enrolled (88% re-treatment, 42% smear positive, 93% pulmonary TB, 74% HIV co-infected). At baseline, 44/88 (50%) MGIT and 28 (32%) LJ cultures were positive with a mean time to positivity of 12.1 (Range 1-42) and 21.5 (Range 7-58) days, respectively. Four percent (3/77) of retreatment patients or 8% of the 38 MGIT+ PCR-confirmed retreatment cases had multi-drug resistant tuberculosis (MDR TB). One MDR TB patient was smear negative and only one MDR patient was identified with LJ. Lower mean hemoglobin at admission was associated with mortality (10.5 vs. 7.5; $p<0.01$; CI 101 9.8-11.0).

Conclusions: The MDR TB burden among the retreatment population in Lilongwe, Malawi is similar to regional estimates by the WHO (7.7% 95% CI 0-18.1). MDR TB patients are not routinely identified with sputum smear or LJ, suggesting more efficient technology should be adopted.

Introduction

Emergence of drug-resistant tuberculosis (TB) raises new challenges for existing TB control programs and may contribute to early mortality, particularly in the setting of HIV co-infection.[1] Mycobacterium tuberculosis (TB) infection and drug susceptibility continue to be difficult to diagnose in resource-limited settings. In Malawi, the annual incidence of TB is estimated at 304/100,0002 and 21,886 new cases were reported in 2009. Approximately 64% of incident TB cases in Malawi are HIV positive.[2]

Recent national statistics of TB drug resistance in Malawi are limited. Based on African regional data, the multidrug-resistant tuberculosis (MDR TB) burden is estimated at 7.7% (95% CI 0.0-18.1) and 1.8% (95% CI 0.0-4.3) in retreatment and first treatment patients, respectively.[3]

The WHO recommends liquid culture and PCR based drug susceptibility testing where available to rapidly diagnose drug-resistant TB.[4]

The Malawi TB program currently uses Lowenstein-Jensen (LJ) solid culture media and direct susceptibility testing which typically require up to eight weeks for results. The use of the liquid culture BACTEC™ Mycobacteria Growth Indicator Tube (MGIT) system and Hain Lifescience Genotype® MTBDRplus assay can reduce the time to detection of resistant strains to less than 14 days (compared to at least 21 days with LJ technique and direct testing).[5-8] The Genotype® MTBDRplus uses PCR to rapidly identify wildtype genes confirming the presence of M. tuberculosis spp. and isoniazid/rifampicin sensitivity as well as gene mutations that confer isoniazid or rifampicin resistance. In a recent meta-analysis evaluating Genotype® MTBDRplus performance, the pooled sensitivity and specificity for 138 detection of rifampin resistance were both 99%, while for isoniazid, it was 96% and 100%, respectively.[9] We evaluated clinical characteristics, yield of solid versus liquid culture, PCR-based drug-resistance profiles, and clinical outcomes of a population of TB inpatients in Lilongwe, Malawi.

Methods

Study setting

The Bwaila inpatient TB ward is located at the Bwaila Hospital in the district of Lilongwe, Malawi. Patients are primarily admitted to the ward from the neighboring Martin Preuss Centre, the Lilongwe district's main outpatient HIV/TB clinic or as transfers from the Kamuzu Central Hospital, that serves the central region of Malawi. Patients may also be admitted from surrounding inpatient and outpatient facilities within the district. In 2008, 428 patients were treated of which 297 (70%) had been previously treated for TB. The remaining were admitted for their first treatment due to severity of illness.

According to Malawi National Guidelines for retreatment patients, sputum should be collected and sent to Lilongwe's Central Reference Laboratory at the Community Health Sciences Unit (CHSU) for LJ culture and direct susceptibility testing for rifampicin,isoniazid, pyrizinamide, and ethambutol resistance where drug containing and drug free media are inoculated directly with a concentrated specimen. Direct susceptibility testing is performed only in retreatment patients as this population has higher risk for drug-resistant tuberculosis. The LJ culture results require at least eight weeks and are recognized to have a low sensitivity and specificity for identifying TB.[10]

While awaiting these results, patients with drug-resistant TB often suffer clinical deterioration on first-line drugs, may transmit resistant strains to other patients, guardians, and health care workers or be discharged without knowledge of their drug susceptibility profiles.[10]

Study population

We conducted a prospective observational cohort study of adult patients admitted to the inpatient TB ward at Bwaila Hospital in Lilongwe, Malawi from January 2010-August 2010. Initially, only retreatment patient were enrolled as only these patients routinely receive drug-susceptibility testing per Malawi National Guidelines. In April 2010, we submitted an amendment to include first treatment patients to determine the prevalence of drug resistance in these patients and improve their clinical care. We also sought to increase the rate of recruitment to achieve an adequate sample size for the time allotted to the study.

First treatment patients were defined as those that had no record of TB treatment in the past. Retreatment patients previously received anti-TB therapy; since past records did not adequately document treatment outcomes, "defaulters," "treatment failures" and "relapsed" patients were grouped in a general category of "retreatment" patients. All patients were >=18 years old. Pregnant women and prisoners were excluded. All patients received TB treatment per Malawi National Guidelines (streptomycin + isoniazid, rifampicin, pyrizinamide and ethambutol for retreatment patients; isoniazid, rifampicin, pyrizinamide and ethambutol for first treatment patients).

Procedures

All participants provided written informed consent. Patient demographics, past medical history, clinical data including HIV status, past and current ART history, and radiographic data were collected at admission on a standardized data form. Alternative diagnoses were evaluated and treated, if identified. Patients were followed for the duration of their stay in the inpatient unit: two weeks for first treatment patients or eight weeks for retreatment patients. All patients with unknown HIV status were offered rapid HIV testing and post-test counseling. Highly active antiretroviral therapy (HAART), if appropriate, was initiated per Malawi National Guidelines.

Patients identified with MDR TB were registered with the National TB Programme and received home-based directly observed second-line treatment with kanamycin, cycloserine, ciprofloxacin and ethionamide.

Specimen collection

At enrollment, we collected three sputum samples, blood for complete blood count (CBC) and alanine aminotranferase (ALT), and if HIV positive, CD4 count and plasma HIV RNA. All samples were transported to UNC project laboratory.

Microbiology

Sputum smears were prepared using auramine-O stain and visualized using fluorescent microscopy. Smear positive samples were defined as those initially observed to contain bacilli through fluorescent microscopy consistent with M.tuberculosis infection. Sputum samples were then cultured on LJ and BACTEC™ MGIT media. Smears from positive cultures were prepared using auramine-O stain and visualized with fluorescent microscopy to differentiate bacilli consistent with TB and contaminant flora. Suspected M. tuberculosis cultures were confirmed using Genotype® MTBDRplus (Hain Lifescience) and resistance profiles to rifampin and isoniazid were determined. Direct susceptibility testing of positive LJ cultures were performed only for patients who had MGIT+ drug-resistant cultures due to human resource, material and cost constraints. Patients who tested negative for Mycobacterium tuberculosis on Genotype® MTBDRplus underwent additional testing with Genotype® Mycobacterium CM (Hain Lifescience)[11] to differentiate between other common Mycobacterial infections. MDR TB patients (resistant to both isoniazid and rifampicin) underwent additional testing with Genotype® MTBDRsl (Hain Lifescience)[12] to identify extensively drug resistant TB (XDR-TB) strains, defined as resistant to isoniazid, rifampicin, any of the fluoroquinolones (ofloxacin or moxifloxacin), and at least one of the three injectable second-line drugs (amikacin,capreomycin or kanamycin).

Statistical analysis

Clinical and laboratory data were double-entered into a customized Microsoft® Access 2007 database. All statistical analyses were conducted using Stata® version 11.0 (Stata Corporation, College Station, Texas, USA). We used Fisher's exact tests and unpaired t-tests to identify patient characteristics associated with mortality or drug resistance.

Ethics review

The study proposal and consent forms were approved by the Malawi National Health Science Research Committee and the University of North Carolina, Chapel Hill Institutional Review Board.

Results

Clinical characteristics

Of 165 patients admitted to the ward during January 2010-August 2010 (68% retreatment, 42% female), 65 (39%) did not meet inclusion criteria: (2 <18 years old; 1 prisoner; 20 first treatment, before amendment; 30 did not consent; 2 too sick to consent; 10 screened, but died prior to enrollment).

One hundred participants met initial inclusion criteria, consented to participate and were enrolled (59% male, 89% retreatment, 39% smear positive, 92% pulmonary TB).

After enrollment, five patients did not submit sputum samples (three died prior to submission). Additionally, 7/61 (11%) smear negative, retreatment patients were determined to be misdiagnosed with TB after enrollment and tuberculosis treatment was discontinued. Alternative diagnoses included residual scarring from previous TB with no new lesions (4), congestive heart failure (1), sarcoidosis (1) and cryptococcal meningitis (1) (Figure 1). Consequently, these 12 patients were excluded from our analysis.

Patient characteristics

(Table 1) Eighty-eight patients were included in the final analysis (57% male, 88% retreatment, 42% smear positive, 93% pulmonary TB).

Nine percent of patients had extrapulmonary manifestations of TB: meningitis (2), pericarditis (4), peritonitis (1) and spinal (1). Ninety-three percent of patients had abnormal findings on chest radiograph. Seventy-four percent were HIV positive and among the positive cases, 64% were on ART at the time of presentation. Seventy per cent of retreatment and all eleven first treatment patients were HIV positive.

Smear and culture results at baseline

Of 88 baseline sputum samples, 44 (50%) were MGIT+ (80% smear positive, 20% smear negative) and 28 (32%) LJ+ (86% smear positive, 14% smear negative) with a mean time to positivity of 12.1 (Range 1-42) and 21.5 (Range 7-58) days, respectively. Sensitivity and specificity of auramine-O smear fluorescent microscopy when compared to MGIT+ culture was 83% and 96%,respectively. Among the 44 MGIT+ cultures at baseline, 38 were confirmed to be M.tuberculosis and 6 were identified as other common Mycobacterial infections using Genotype® Mycobacterium CM: M. fortuitum (2), M. interjectum (1), and M.intracellulare (3). M. tuberculosis drug susceptibility profiles Among the baseline TB cultures, 33/38 (87%) were pan-sensitive, one had isoniazid mono-resistance and one had rifampicin mono-resistance. Three patients had MDR TB. Only one MDR-TB patient was identified with LJ and

direct susceptibility testing. The prevalence of MDR-TB was 3/77 (4%) among clinically diagnosed retreatment patients and 3/38 (8%) among MGIT+ PCR-confirmed retreatment cases. All MDR TB cultures underwent further testing with Genotype® MTBDRsl. All three had additional resistance to ethambutol,but no extensively drug-resistant (XDR) TB was detected. Four of the five drug resistant patients were HIV co-infected.

Table 1. Demographic data and clinical characteristics at admission (N=88)

Baseline Characteristics	All Patients (N=88)	Retreatment (N=77)	First treatment (N=11)
Mean age, years (range)	38.8 (18-75)	38.1 (18-75)	43.5 (28-75)
Female (%)	38 (43)	30 (34)	8 (73)
Smear positive (%)	37 (42)	31 (40)	6 (55)
Pulmonary TB	82 (93)	72 (94)	10 (91)
Extrapulmonary TB (% total)	8 (9)	6 (8)	2 (18)
Meningitis (% extrapulmonary)	2 (33)	1 (20)	1 (50)
Pericarditis	4 (50)	3 (50)	1 (50)
Peritonitis	1 (17)	1 (20)	-
Spinal	1 (17)	1 (20)	-
Abnormal Chest X-ray (%) (N=76 Xrays, 67 retreatment; 9 first treatment)	71 (93)	63 (94)	8 (89)
Unilateral infiltrate	10 (14)	10 (16)	-
Bilateral infiltrate	55 (77)	48 (76)	7 (88)
Adenopathy	31 (44)	28 (44)	3 (38)
Miliary pattern	4 (6)	3 (5)	1 (13)
Cavitary lesion	23 (32)	20 (32)	3 (38)
Pleural effusion	9 (13)	9 (14)	-
Suspected pericardial effusion*	4 (6)	3 (5)	1 (13)
HIV positive	65 (74)	54 (70)	11 (100)
On ART (% HIV+)	44 (68)	43 (80)	1 (9)
Median CD4 count (IQR)	135 (67.5-250.5)	144 (69-254)	125 (46-154)
Median log HIV RNA copies/mL (IQR)	2.60 (2.60-5.40)	2.60 (2.60-5.18)	2.81 (2.60-5.57)
ART failure (% on ART)**	2 (5)	2 (5)	0

Unconfirmed infection

In 44/88 (50%) patients, there were no positive cultures. 42/51 (82%) smear negative patients had unconfirmed infection. The remaining two smear positive samples had contaminated cultures. Most unconfirmed infections occurred in HIV/TB co-infected patients (36/42, 85%).

TB clinical management and outcomes

All pan-sensitive patients continued first-line TB therapy. All MDR patients were discharged home to start second-line TB therapy administered and monitored by a Malawi National TB Programme officer within seven days of diagnosis. Isoniazid mono-resistance was treated with an increased dose of isoniazid (100 mg/kg) and continuation of rifampicin, pyrizinamide and ethambutol. Rifampicin mono-resistance was treated with isoniazid, pyrizinamide and ethambutol alone. Culture-negative patients were presumed to have TB based on clinical presentation and continued on their initially assigned therapy. Patients identified with Mycobacterial infections other than tuberculosis were continued on their anti-TB regimen (5 retreatment, 1 first treatment) as poly-antimicrobial therapy may benefit patients with disseminated infection.[13] Further, mixed infection with Mycobacterium tuberculosis could not be ruled out.[14]

At eight weeks, of the 88 patients enrolled, 81 (85%) completed follow-up, 5 died, and 2 transferred care. Mortality was 4% (3/77) and 18% (2/11) for retreatment and first treatment patients, respectively. Lower mean hemoglobin at admission was associated with mortality (10.5 vs. 7.5; $p<0.01$; CI 9.8-11.0). No other evaluated factors were associated with drug resistance or mortality (Table 2 and 3).

Table 2. Associations with any form of drug resistance*

	Pan-sensitive (N=83)	Any drug-resistance (N=5)
Female (%)	37 (45)	1 (20)
Mean Age, years	38.6	42.6
Mean BMI at admission	18.7	17.7
First treatment (%)	11 (13)	0
Retreatment (%)	72 (87)	5 (100)
Abnormal X-ray (%)	66 (80)	5 (100)
Pulmonary TB (%)	76 (92)	5 (100)
Extrapulmonary TB (%)	6 (7)	0
Smear positive at admission (%)	34 (41)	3 (60)
HIV positive (%)	61 (73)	4 (80)
Not on ART at admission (% HIV+)	8 (13)	0
Mean hemoglobin at admission (g/dl)	10.4	10.2
Median CD4 count at admission	194.3	145.5

***No associations approached statistical significance.**

Table 3. Associations with mortality*

	Alive at Discharge (N=83)	Died before completing follow-up (N=5)
Female (%)	35 (42)	3 (60)
Mean Age, years	38.2	48.3
Mean BMI at admission	18.5	21.1
First treatment (%)	9 (11)	2 (40)
Retreatment (%)	74 (89)	3 (60)
Abnormal X-ray (%)	67 (81)	4 (80)
Pulmonary TB (%)	78 (94)	4 (80)
Extrapulmonary TB (%)	5 (6)	1 (20)
Smear positive at admission (%)	33 (40)	4 (80)
HIV positive (%)	62 (75)	3 (60)
Not on ART at admission (% HIV+)	7 (11)	1 (33)
Mean hemoglobin at admission (g/dl)	10.6	7.5
Median CD4 count at admission	194.7	84

*Only the association with mean hemoglobin at admission was statistically significant, p<0.0

Discussion

Implementation of florescent microscopy and rapid TB diagnostic and drug susceptibility testing may optimize national TB program operations throughout resource-limited settings. This is the first study to date in Malawi that compares LJ and MGIT culture systems and uses PCR-based susceptibility testing to estimate prevalence of drug resistant Mycobacterium tuberculosis. Consistent with other studies, fluorescent microscopy had high sensitivity compared to MGIT culture and MGIT culture provided better yield more rapidly when compared to LJ.

Within the Malawi TB program, clinicians rely on clinical presentation, sputum smear and chest radiography. Even within our small study, the clinical implications of improved diagnostics were evident, particularly among smear negative clients. MGIT cultures confirmed TB in a significant number of smear negative patients. Further, MGIT results were more rapid and allowed the use of PCR-based drug resistance testing. Notably, all patients with confirmed drug resistance were switched to appropriate therapy within seven days of their initial TB diagnosis, potentially improving outcomes and preventing transmission of drug-resistant strains. In the absence of liquid culture and PCR, 66% of patients with multi-drug resistant TB would have been missed.

We report an MDR TB prevalence of 3/77 (4%) among clinically diagnosed retreatment patients and 3/38 (8%) among MGIT+ retreatment cases. This is similar to WHO estimates based on regional modeling (7.7% CI 0.0-18.1) (WHO). No drug resistance was identified among first treatment patients in our study (WHO regional estimate of MDR among first treatment patients: 1.8% (0.0-4.3)). Additionally, no XDR TB was detected.

We acknowledge certain limitations to our study. A significant proportion (25%) of patients were admitted to the ward and received treatment per Malawi National Guidelines, but were not interested in participating in the study. As this was a pilot cohort, the sample size goal of 100 patients was designed to gather preliminary data for further investigation of drug-resistant TB prevalence among inpatients and outpatients in Lilongwe, Malawi. However, twelve of these patients were excluded from analysis after enrollment. Given our small evaluable sample size, it was difficult to determine

any associations between patient characteristics and drug-resistance or mortality. Notably, patients that were too ill or did not survive to enroll in the study may have contributed to an underestimate of MDR prevalence and prevented complete evaluation of factors associated with mortality. While we identified some misdiagnosed cases of smear negative TB in our study operations, additional misdiagnosed cases may have been included in the sample due to challenges in diagnosing TB.

A significant proportion of clinically diagnosed TB patients had positive non tubercular Mycobacterial cultures. While these organisms can act as pathogens or colonizers, we suspect that they may have contributed to our patient's respiratory symptoms and determined that continuing anti-TB therapy was appropriate. We could not rule out disseminated infection nor drug susceptibility of these cultures.

In summary, we demonstrate that PCR-based technology can be applied in Malawi to more effectively diagnose TB, determine drug susceptibility profiles, and impact clinical care when compared to LJ and direct susceptibility testing. We recognize that assays like Hain Genotype® MTBDRplus require a sufficient budget to purchase equipment, build bio-containment infrastructure and provide highly-skilled laboratory technicians, which is not easily availably in resource-limited settings. This tool would be best suited for Central laboratories and hospitals in Malawi. However, given the high sensitivity of florescent microscopy and its ease of use, this TB diagnostic tool can more readily be implemented at diagnostic facilities to improve TB diagnosis.However, cost of equipment and trained technicians may continue to be a limiting factor.

It is important to consider other potentially applicable technologies for rapid TB diagnosis and susceptibility testing in routine clinical settings in Malawi. Most recently, the Gene X-pert (Cepheid) offers a model for sensitive, rapid and automated molecular testing for rifampicin-resistance in resource-limited settings and has been endorsed as a point-of-care tool.[15,16]

In contrast to the Hain Genotype® MTBDRplus, it can be performed by lower cadre staff without bio-containment facilities. Additional implementation studies with such point-of-care devices are needed to determine the feasibility of their standardized use in existing TB control programs.

In conclusion, the MDRTB prevalence among the retreatment population in our Malawian inpatient cohort is similar to WHO models based on regional estimates. The use of smear and LJ culture technique alone failed to rapidly diagnose TB, determine drug susceptibility profiles and impact clinical care suggesting that more efficient, PCR-based diagnostic technology should be adopted.

Acknowledgements

We are grateful for support from the University of North Carolina Center for AIDS Research (P30 AI50410), the International Union Against Tuberculosis and Lung Disease and the National Institutes of Health Office of the Director, Fogarty International Center, Office of AIDS Research, National Cancer Center, National Eye Institute, National Heart, Blood, and Lung Institute, National Institute of Dental & Craniofacial Research, National Institute On Drug Abuse, National Institute of Mental Health, National Institute of Allergy and Infectious Diseases Health, and NIH Office of Women's Health and Research through the International Clinical Research Scholars and Fellows Program at Vanderbilt University (R24 TW007988) and the American Relief and Recovery Act.

We would like to offer our gratitude to the administration and staff of the Malawi National TB Programme, Community Health Sciences Unit, Martin Preusse Center, and the Bwaila TB Ward, without whom this study would not have been possible. CKV and DLK were recipients of NIH-Fogarty International Clinical Research Scholarships (2009-10).

References

1. Brust JC, Ghandi NR, Carrara H, et al. High treatment failure and default rates for patients with multidrug-resistant tuberculosis in KwaZulu-Natal, South Africa, 2000-2003. Int J Tuberc Lung Dis 2010; 13:413-9.

2. WHO. Tuberculosis country profile: Malawi. Accessed on July 16, 2011.https://extranet.who.int/sree/Reports?op=Replet&name=/WHO_HQ_Reports/G2/PROD/EXT/TBCountryProfile&ISO2=MW&outtype=html.

3. WHO. Multidrug and extensively drug-resistant tuberculosis (X/MDR-TB): 2010 Global Report on Surveillance and Response. 2010.

4. WHO. Molecular line probe assays for rapid screening of patients at risk of multidrug-resistant tuberculosis (MDR-TB). Geneva: World Health Organisation.2008.

5. Chien HP, Yu MC, Wu MH, et al. Comparison of the BACTEC MGIT 960 withLöwenstein-Jensen medium for recovery of mycobacteria from clinical specimens. Int J Tuberc Lung Dis 2000; 4: 866-870.

6. Idigoras P, Beristain X, Iturzaeta A, et al. Comparison of the automated nonradiometric Bactec MGIT 960 System with Löwenstein-Jensen, Colletsos, and Middlebrook 7H11 solid media for recovery of mycobacteria. Eur J Clin Microbiol Infect Dis 2000; 19: 350–354.

7. Lu D, Heeren B, Dunne WM. Comparison of the automated Mycobacteria Growth Indicator Tube System (BACTEC 960/MGIT) with Löwenstein-Jensen Medium for recovery of mycobacteria from clinical specimens. Am J Clin Pathol 2002; 118: 542-545.

8. Mueller DH, Mwenge L, Muyoyeta M, et al. Costs and cost-effectiveness of tuberculosis cultures using solid and liquid media in a developing country. Int J Tuberc Lung Dis 2008; 12:1196-1202.

9. Ling DI, Zwerling AA, Pai M. GenoType MTBDR assays for the diagnosis ofmultidrug-resistant tuberculosis: a meta-analysis. Eur Resp J 2008; 32:1165-1174.

10. Harries AD, Kamenya A, Namarika D, et al. Delays in diagnosis and treatment of smear-positive tuberculosis and incidence of tuberculosis in hospital nurses in Blantyre, Malawi. Trans R Soc Trop Med Hyg 1997; 91:15-7.

11. Couto I, Machado D, Viveiros M, et al. Identification of nontuberculous mycobacteria in clinical samples using molecular methods: a 3-year study. Clin Microbiol Infect 2010; 16:1161-1164.

12. Kiet VS, Lan NT, An DD, et al. Evaluation of MTBDRsl test for detection of second-line-drug resistance in Mycobacterium tuberculosis. J Clin Microbiol 2010; 48:2934-2939.

13. Gordin FM, Sullam PM, Shafran SD, et al. A randomized, placebo-controlled study of rifabutin added to a regimen of clarithromycin and ethambutol for treatment of disseminated infection with Mycobacterium avium complex. Clin Infect Dis. May 1999;28(5):1080-5

14. Mallard K, McNerney, Crampin AC, et al. Molecular 459 Detection of Mixed nfections of Mycobacterium tuberculosis Strains in Sputum Samples from Patients in Karonga District, Malawi. J Clin Microbiology 2010; 48: 4512-4518.

15. Van Rie A, Page-Shipp L, Lesley S, et al. Xpert® 463 MTB/RIF for point-of-care diagnosis of TB in high-HIV burden, resource-limited countries: hype or hope? Expert Rev Mol Diagn 2010; 10:937-946.

16. Boehme CC, Nabeta P, Hilleman, et al. Rapid Molecular Detection of Tuberculosis and Rifampin Resistance. N Engl J Med 2010; 363:1005-1015.