JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

A suggested algorithm for detection of multi drug-resistant tuberculosis in Zimbabwe

Beauty Makamure[1], Salome Makumbirofa[1], Tsitsi Bandason[1], Paul Leccese[2], Reggie Mutetwa[1], Val Robertson[3], Peter Mason[1]

[1] Biomedical Research and Training Institute (BRTI), Harare, Zimbabwe
[2] University of Colorado School of Medicine, Aurora, Colorado, United States
[3] Department of Medical Microbiology, University of Zimbabwe, Harare, Zimbabwe

Abstract

Introduction: Rapid genotypic and phenotypic methods for multi-drug-resistant-tuberculosis (MDR-TB) detection are now widely available. Zimbabwe adopted the use of GeneXpert-MTB/RIF, microscopic-observation-drug-susceptibility-assay (MODS) and Mycobacteria-Growth-Indicator-Tube (MGIT) drug-susceptibility-testing (DST). Data is limited on the ideal combination of use of these methods in resource limited settings.

Methodology: Between August 2014 to July 2015, 211 sputa from MDR-TB suspects were tested with GeneXpert-MTB/RIF, MODS, manual-MGIT and Lowenstein-Jensen (LJ)-DST to determine diagnostic accuracy and turnaround-time (TAT), with LJ-DST as the gold standard. A performance score ranking table for diagnostic accuracy, TAT, costs, facilities and expertise requirements, was used to determine the most favourable tool.

Results: GeneXpert-MTB/RIF sensitivity was 96% (95%CI:80-100) and specificity was 95% (95%CI:90-97). MODS sensitivity was 88% (95%CI:68-97) and specificity was 97% (95%CI:87-100). Manual MGIT-DST had slightly lower sensitivity of 80% (95%CI:59-93). Median time to detection of MDR-TB was <1 day (IQR:0-0) for Xpert, 14 days (IQR:11-31) for MODS, 21 days (IQR:7-22) for MGIT-DST and 28 days (IQR:25-28) for LJ-DST. Operational costs for MODS, MGIT-DST, and GeneXpert-MTB/RIF were $21.20, $27.52 and $39.76 respectively. From a summation of scores including facility and expertise requirements per diagnostic technique, GeneXpert-MTB/RIF was the most favourable tool, followed by MODS and MGIT-DST.

Conclusions: For best scale-up of MDR-TB diagnosis in Zimbabwe, GeneXpert-MTB/RIF can be used for rapid detection of TB in smear negative cases, RIF-susceptibility for early treatment initiation and probable MDR-TB. MODS can rapidly confirm probable MDR-TB detected by GeneXpert-MTB/RIF, manual-MGIT can provide early results for susceptibility to other antibiotics, with affordable costs, with LJ-DST confirming discordant DSTs.

Key words: Tuberculosis; MDR-TB diagnosis; sensitivity; specificity; turnaround time; Zimbabwe.

J Infect Dev Ctries 2017; 11(8):611-618. doi:10.3855/jidc.8009

(Received 16 December 2015 – Accepted 22 July 2016)

Introduction

Tuberculosis (TB) kills an estimated 2 million people each year worldwide, making it the leading cause of infectious disease deaths amongst young adults in much of the world [1]. The emergence of MDR-TB strains has become an increasing concern [2] particularly in areas with high TB and Human Immunodeficiency Virus (HIV) burdens where resources for detection and treatment may be limited.

More than 75,000 new cases of multi drug resistant TB (MDR-TB) are estimated to occur each year in Africa, with South Africa having the highest MDR-TB burden (incidence of 25 cases per 100,000 people) [3,4]. A recent prospective study in Zimbabwe showed 25% of retreatment TB cases had an MDR-TB infection [5] though retreatment cases are not generally representative of TB, and there is an urgent need for a national survey to determine the prevalence of MDR-TB in a more random sample of the population.

Laboratory delays in the detection, identification and reporting of MDR-TB cases contributes at least in part to the spread of the disease [6]. To reduce diagnostic delays, the World Health Organization (WHO) recommends empirical therapy of all new TB cases using first-line drugs, with a switch to more appropriate treatment if subsequent tests show infection with MDR-TB [7]. The WHO also recommends that TB suspects at high risk of MDR-TB should be screened for RIF resistance using rapid diagnostic tests, [8] since this is highly indicative of MDR-TB. Both of these

approaches require laboratory confirmation of an MDR-TB infection to ensure effective treatment, and this poses challenges in resource limited settings.

The use of solid Lowenstein-Jensen (LJ) media which is currently regarded as the gold standard for DST, is relatively inexpensive, but it requires specialized laboratories and well-trained and experienced staff. The prolonged detection time (8-12 weeks) for *Mycobacterium tuberculosis* [MTB] growth, detection, identification and drug susceptibility testing [DST]) means, however, that patients may be treated inappropriately, leading to the spread of drug resistant strains in the community.

In the past 5 years, rapid diagnostic genotypic methods (particularly GeneXpert-MTB/RIF) have become widely available even in countries with limited resources. In addition, rapid phenotypic methods for detecting resistance such as Microscopic Observation Drug Susceptibility Assay (MODS) have been developed. Currently in Zimbabwe smear microscopy (SM), solid (LJ) culture media and liquid culture using Mycobacteria Growth Indicator Tube (MGIT) are used for routine detection of TB, with MGIT DST, MODS, GeneXpert-MTB/RIF and Hain Line probe assays being used to detect MDR-TB. Each technique has advantages and limitations, particularly with regards to cost, infrastructural requirements and time to detection. Using GeneXpert-MTB/RIF, the median time from sample collection to detection of resistance to rifampicin is 2 days, compared with 7 days using MODS, 24 days using MGIT-DST and many weeks using LJ-DST [9,10]. GeneXpert-MTB/RIF however detects resistance only to RIF and so is only a surrogate marker for MDR-TB [11] and for confirmation, other tests such as culture are required. Moreover, recent evidence suggests that its value is not the same in all settings [12]. MODS can be used to determine susceptibility to both RIF and INH, enabling confirmation of an isolate as MDR-TB, but resistance to other antibiotics included in standard first line treatment is not reliable [9,10]. The MGIT-DST system, shows high correlation with conventional solid culture DST for detection of resistance to both first and second-line anti-TB drugs, but it is more expensive, limiting its availability in developing countries [13,14].

A review by Myo Nyein Aung *et al* discussed a new TB diagnostic algorithm that skips traditional smear and solid culture diagnostic methods preferring the WHO recommended GeneXpert algorithm, which was found to have costs which are less than either smear-Chest X-Ray (CXR) or smear-CXR-culture algorithms. Furthermore, culture and GeneXpert algorithms have been found to be more cost effective in reducing mortality than the current practice (symptoms screening, sputum smear, and chest radiography) [15,16].

We have used data from a study of MDR-TB in suspect cases to develop an effective algorithm for MDR-TB diagnosis in Zimbabwe using combinations of GeneXpert-MTB/RIF, MODS, MGIT and LJ-DST that were appropriate to laboratories in countries with limited TB diagnostic facilities.

Methodology

Data on sensitivity and specificity of the different tests were obtained from a cross sectional study conducted in Harare, between August 2014 to July 2015, where we tested 211 sputum samples from MDR-TB high risk patients (TB symptoms with at least one of: previously confirmed MDR-TB, failure to convert after at least two months therapy, treatment failure, return after default, relapse after completion of treatment or contacts of known MDR-TB cases). This sample was adequate to achieve a precision of 20% for sensitivity and 3% for specificity based on MDR-TB prevalence of 5.8%, LJ sensitivity of 84% and specificity of 96%, where the minimum sample size required was estimated to be 200. Samples were screened from MDR-TB high risk patients' spot and morning sputum samples, spontaneously expectorated by front loading, on the day of enrolment and sent to the laboratory for routine MDR-TB diagnosis. Samples were sent to the laboratory under cold chain, in cooler boxes with temperatures maintained at 2ºC to 8ºC using ice packs.

We examined sputa as detailed below using smear microscopy, GeneXpert-MTB/RIF, MODS, MGIT (using manual rather than automated detection) and LJ DST, for detection of MDR-TB. The HIV status of the patients was indicated on the laboratory request form by the referring facility but was not confirmed in our laboratory.

Study Setting

The samples were processed at the Biomedical Research and Training Institute (BRTI) TB Laboratory in Harare, Zimbabwe. The laboratory is ISO 15189 accredited and performs external quality assurance for smear microscopy, liquid and solid culture and DST using solid culture with the National Health Laboratory Services of South Africa and the Centre for American Pathology.

Laboratory Methods

Three aliquots of expectorated sputum were obtained from homogenized spot and morning sputa. All sputa were decontaminated, decongested and concentrated using standard laboratory methods. The first aliquot was submitted for smear microscopy, MGIT and solid LJ culture, the second aliquot was used for GeneXpert-MTB/RIF testing and the third aliquot was used for MODS testing as shown in Figure 1. Both Ziehl Nielsen (ZN), (Gainland Chemical Company, Sandy croft, Deeside, UK) and Auramine (Park Scientific, Northampton, UK) staining were used for smear microscopy and slides were examined by laboratory scientists and trained microscopists. MGIT tubes were examined daily for up to 40 days for evidence of growth, and confirmed as mycobacteria using ZN microscopy. MGIT cultures that had a mixture of mycobacteria and other bacterial contamination from 21 to 40 days were re-decontaminated and re-cultured. Solid LJ cultures were monitored for up to 12 weeks before being diagnosed as negative. Commercial kits were used for MODS (TB MODS Kit, Hardy Diagnostics, Santa Maria, USA) and GeneXpert-MTB/RIF (Cepheid, Sunnyvale, USA), using the manufacturer's instructions.

All MTB isolates, from whichever source, were tested for drug resistance to rifampicin, isoniazid, ethambutol and streptomycin using MGIT-DST and the absolute concentration measurement on LJ media prepared in house in the BRTI TB laboratory.

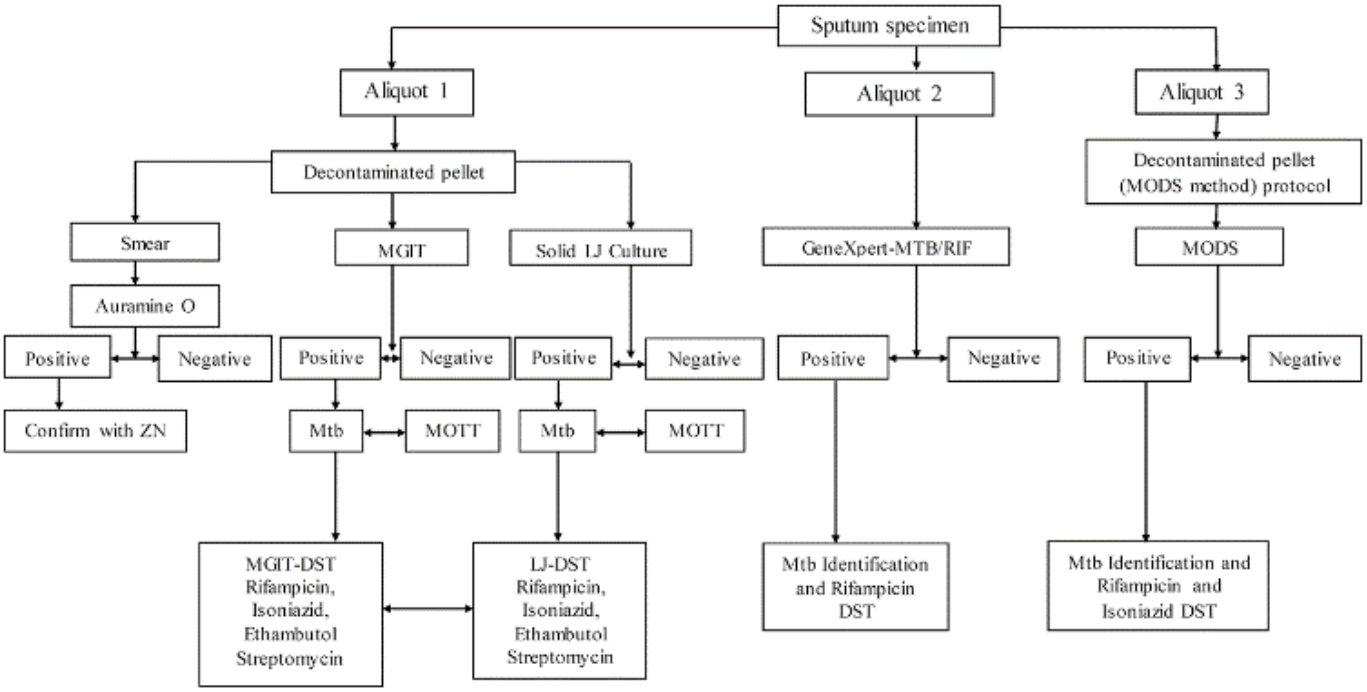
Test performance

The three DST tests (GeneXpert MTB/RIF, MODS and MGIT) were ranked in order of performance (from 1-3) using the categories of sensitivity, specificity, turnaround time (being the time from setting the test to having the results), estimated cost and infrastructural demands [17]. Scores were ranked based on Zeman DH principle and the total ranking scores were used to assess overall performance [18,19]. Costs were estimated using data collected in Zimbabwe by Leccese P, Makamure B, Dowdy D.D, and Metcalfe J.Z. A Cost-Consequence Model Comparing GeneXpert-MTB/RIF and Microscopic Observation Drug Susceptibility Testing for the Diagnosis of Suspected Drug-Resistant Pulmonary Tuberculosis in Adults in Harare, Zimbabwe (presented at a Poster session at American Thoracic Society International Conference; Denver, CO USA. 2015, 15-20). Cost estimates were generated through consideration of all substantial resource inputs necessary to perform each diagnostic test, including laboratory overhead costs, personnel costs, laboratory equipment, consumable supplies, test reagents, sample collection, transportation, and sample decontamination. All costs are reported in 2014 United States dollars.

Ethical approval

Ethical approval was obtained from the BRTI Institution Review Board (IRB), the Joint Research Ethics Committee (J-REC) and the Medical Research Council of Zimbabwe (MRCZ).

Figure 1. Specimen flow chart.



Statistical analysis

Data were analyzed using STATA version 13 (StataCorp, College Station, Texas, USA). For the primary analyses of sensitivity, specificity, positive predictive value, and negative predictive value of the diagnostic tests, LJ-DST was used as the gold standard. A composite reference range was described as any positive culture result obtained by either MGIT or LJ culture. Descriptive statistics and proportions were calculated to describe the demographic characteristics of the participants. Median time to MTB or MDR –TB detection was calculated and Kaplan Meier survival curves were used for length of time to detection. Although the aim of the study was not to assess performance of the tests, Mann–Whitney (Wilcoxon) non-parametric test was used to compare median time to detection and McNemar chi-square test was used to compare sensitivity and specificity of the diagnostic tests.

Results

Demographic Characteristics

Details of the demographic characteristics are shown in Table 1. The median age of the participants who provided the specimens was 38 years (IQR; 30-47), and of those who were diagnosed with MTB the great majority were over 25 years.

Diagnostic Accuracy of the different tools to detect MDR-TB

The prevalence of MDR-TB in the sample was 13% (95%CI:8-18%) based on LJ-DST as the gold standard. GeneXpert-MTB/RIF had 96% sensitivity (95%CI:80-100) and 95% specificity (95%CI:90-97) followed by MODS with a sensitivity of 88% (95%CI:68-97) and specificity of 97% (95%CI:87-100). Manual-MGIT had a lower sensitivity of 80% (95%CI:59-93) but higher specificity of 99% (95%CI:96 -100). There was no significant differences in the performance of the different detection methods between specimens from HIV positive and HIV negative patients ($p > 0.05$) (Table 2).

Time to Detection of MDR-TB

The median turnaround time of MDR-TB detection, regardless of HIV status, was shortest using GeneXpert-MTB/RIF (<1 day, IQR 0 - 0). For MODS the median turnaround time was14 days, (IQR:11-31) and the time increased significantly to 19 days (IQR:13-26) among HIV positives ($p = 0,009$). The median turnaround time using other tools was significantly longer, being 21 days (IQR:7-22) for MGIT and 28 days, (IQR: 25-28) for LJ-DST. Furthermore, median time to obtaining an MDR result from the date a sample was received was also shortest using GeneXpert- MTB/RIF (1 day; IQR:1

Table 1. Characteristics of Study population

Characteristics		**N=211**	**%**
Gender	Male	124	58.8%
	Female	87	41.2%
HIV status	Positive	133	63.0%
	Negative	55	26.1%
	Unknown	23	10.9%
Inclusion criteria	New MDR-TB diagnosis cases (confirmed by laboratory tests).	14	6.6%
	Failure to convert after 2 months of Category 1 treatment	1	0.5%
	Failure of Category 1 treatment	47	22.3%
	Return after default	14	6.6%
	Relapse	42	19.9%
	Failure to convert after 3months of Category II treatment	0	0.0%
	Failure of Category II treatment	14	6.6%
	Previously treated with 2nd line drugs (or on treatment)	47	22.3%
	DR-TB Contacts	32	15.2%
	New MDR-TB diagnosis cases (confirmed by laboratory tests).	14	6.6%
MTB	Yes	71	33.6%
	No	139	65.9%
	Contaminated	1	0.5%
		n=71	
MTB by age (years)	≤15	0	0.0%
	16-25	6	8.4%
	26-35	19	26.7%
	36-45	27	38.0%
	>45	19	26.7%

Table 2. Ability of tools to detect MDR-TB by HIV Status. LJ-DST was used as the gold standard.

	GeneXpert-MTB/RIF		MODS		Manual-MGIT-DST	
	HIV+	HIV-	HIV+	HIV-	HIV+	HIV-
Sensitivity (95% CI)	100(81-100)	89(52-100)	65(38-86)	88(47-100)	77(50-93)	88(47-100)
Specificity (95% CI)	94(88-98)	93(82-99)	100(97-100)	98(88-100)	99(95-100)	98(88-100)
PPV* (95%CI)	71(49-87)	73(39-94)	100(72-100)	88(47-100)	93(66-100)	88(47-100)
NPV(95%CI)	100(97-100)	98(88-100)	95(89-98)	98(88-100)	97(91-99)	98(88-100)

* PPV = Positive Predictive Value, NPV=Negative Predictive Value.

- 2), followed by MODS (17 days; IQR: 12-26) and MGIT-DST (39 days; IQR: 28-48). For LJ-DST the median time from receipt of sample to MDR result was 79 days (IQR: 64-96).

Operational costs

Operational cost per sample tested for MODS was $21.20 and $27.52 for manual MGIT-DST. GeneXpert had the highest cost of $39.76.

Ranking score

Based on a summation of the ranking of each of the selected diagnostic tool performance GeneXpert-MTB/RIF proved to be the best tool for MDR-TB diagnosis, followed by MODS, and MGIT-DST (Table 3).

Discussion

The rapid and reliable detection of MDR-TB is a key element in preventing the spread of infections with high morbidity and mortality in a community, [8] and this is particularly the case in resource-limited settings that have high HIV and TB burdens.

Smear microscopy is rapid and is undoubtedly the most inexpensive method used to detect mycobacteria in sputum samples, particularly from HIV-negative patients. Patients with positive microscopy can be put on treatment the same day, reducing the spread of TB in community settings. Microscopy however has a number of limitations – its sensitivity is low and especially in paucibacillary infections which are the most common in HIV-positive patients [20,21]. As many TB patients in southern Africa are co-infected with HIV, this limits the reliability of microscopy as a diagnostic tool. Secondly, microscopy cannot be used to identify mycobacteriaceae MTB, and it gives no indication of drug susceptibility. Its use is therefore limited to screening. with particular value at primary care level, where it can be used to ensure that all patients with suspect mycobacteria in sputum samples are put onto first line TB treatment without delay.

GeneXpert-MTB/RIF can be used for rapid detection of TB in smear negative cases, provide Rif susceptibility for positive cases so that they receive correct treatment early and rapid detection of resistance to rifampicin (probable MDR-TB) [22]. Early detection of MDR-TB will enable switching treatment to second line drugs early in cases where first line treatment had been initiated. GeneXpert had the highest sensitivity and specificity and the best tool, based on the findings of this study. However, it is expensive and requires moderate levels of infrastructure (such as an air-conditioned room and reliable power supplies). That may not be suitable for primary care centres [19]. The main limitation of GeneXprt MTB/RIF is that it cannot be reliably used to detect resistance to first-line

Table 3. Comparison of Sensitivity, Specificity, Turnaround Time, Costs, facility and expertise requirements of GeneXpert-MTB/RIF, MODS, MGIT-DST using LJ-DST as the Gold Standard.

Diagnostic tool	GeneXpert-MTB/RIF	MODS	Manual MGIT DST
Sensitivity	96%	88%	80%
Sensitivity ranking	**1**	**2**	**3**
Specificity	95%	97%	99%
Specificity ranking	**3**	**2**	**1**
Median Turn Around Time	<1	17	21
TAT ranking	**1**	**2**	**3**
Cost	$39.76	$21.20	$27.52
Cost ranking	**3**	**1**	**2**
Cat 3& Scientific requirement	No	Yes	Yes
Cat 3 Requirement ranking	**1**	**2**	**2**
Scientist requirement	No	Yes	Yes
Scientist requirement Score	**1**	**2**	**2**
Total score from ranks	**10**	**11**	**13**

antibiotics other than RIF, and so does not detect true MDR-TB infections. For this reason, culture is required and the three main assays available are MODS, MGIT and solid LJ-DST. Of these three assays, MODS has the the shortest median turnaround time, is less expensive than MGIT [10] and can be used to detect resistance to both RIF and INH. This was confirmed by our findings in that MODS had the shortest turnaround time (14 days) and had the least operational costs per sample tested ($21.20, $27.52 and $22.35. MODS is however not sensitive enough using drugs other than INH and RIF to give a full panel of first-line drug resistance. Due to this reason, MGIT-DST is reliable with a reasonable turnaround time (21 days) and if a manual reader is used, costs within range of solid LJ ($27.52 in our study) and is easy to operate [13,23,24]. Including automatic reading of tubes into the MGIT assay adds considerably to the costs and has advantage only in settings where there is a high flow of specimens through the laboratory. All of these assays, however, require safe facilities and highly skilled personnel, suggesting they are best placed at tertiary levels of care [1,19]. Solid LJ-DST is still regarded as the standard by which to compare the reliability of other tests [25].

We note that there were some limitations to this study. Our assessment did not include the actual work load associated with the use of each of the diagnostic tools which might have given an overestimate or underestimate of the total score for the diagnostic tools.

The cost of testing is an extremely important consideration in resource limited settings [26] and requires factorizing the cost of test materials, labour and training costs (related to both complexity of the test and turnaround time), and infrastructural costs (including instrument purchase and maintenance costs, useful life of instruments, and costs of specialised facilities, such as the need for high levels of laboratory safety). For the purpose of this study we used an analysis of costs presented at an international conference on pulmonary, critical care, and sleep medicine held in Denver, USA.

We also note that our sample size was small (fixed) as there were limited funds and set timelines for completion of the study, which means that our findings cannot be generalised. The small sample size also resulted in a small number of MDR-TB cases being detected. A multi-centre study would be suggested to improve the statistical power and generalizability of the findings.

Conclusion

For best scale up of drug resistance TB case finding in Zimbabwe GeneXpert-MTB/RIF was the most reliable method for detecting MDR-TB in sputum samples, followed by MODS and MGIT-DST. Solid LJ DST was the gold standard. From these findings, we suggest that an ideal testing algorithm and the combinations of the diagnostic tools at different facility levels would be in the order shown in Figure 2 and 3, respectively.

We also recommend that a combination of HIV-testing and microscopy be used at primary care level to ensure that those who are sputum positive receive first line treatment without delay. Sputa should then be transported to the second level (usually District or Provincial laboratories) with moderate levels of infrastructure, where confirmation using GeneXpert MTB-RIF can be done. This gives the opportunity to

Figure 2. Recommended Testing Algorithm.

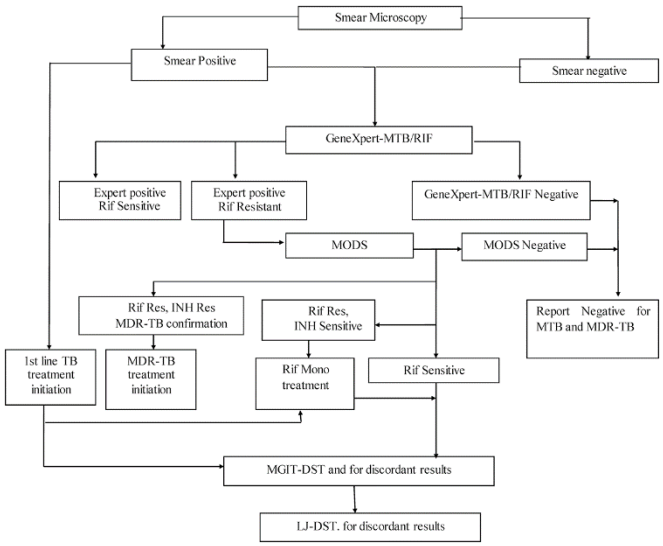
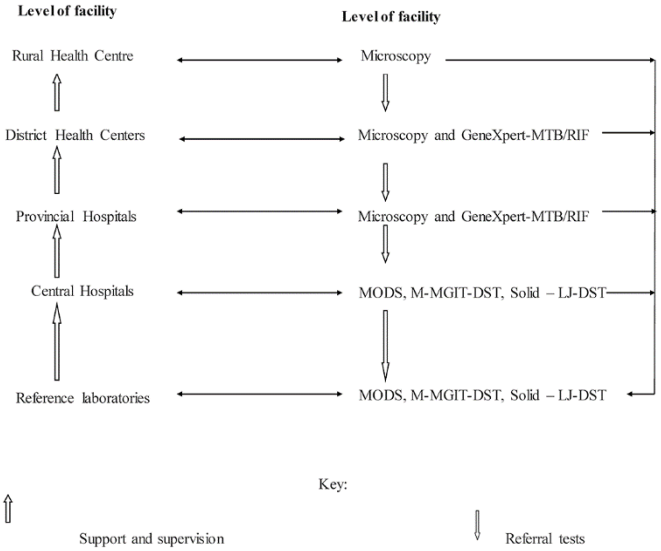


Figure 3. Recommendations for tests and test combinations for the different levels of Health facilities available in Zimbabwe.



detect infections in microscopy-negative, especially HIV positive cases, and to confirm RIF susceptibility in microscopy-positive cases. The first line treatment initiated at primary care level can be continued, and treatment can be initiated for those diagnosed with TB using GeneXpert MTB/RIF. In cases where GeneXpert detects RIF resistance, treatment could be switched to second line drugs while a possible diagnosis of MDR-TB is investigated further, at tertiary laboratories, where MODS and/or MGIT should be available. Finally, solid LJ DST can remain only as a reference standard for confirming any discordant DST results. Supervision and support should be cascaded from the top to the bottom level to ensure that quality standards and continuity of services are maintained.

This algorithm, which includes addition of MODS and manually read MGIT-DST to GeneXpert MTB/RIF Assay, will bring rapid detection of MDR-TB, prompt effective treatment of patients with MDR-TB, will be cost effective in reducing mortality, risk of transmission and early detection of patients requiring second-line treatments.

Acknowledgements

We would like to thank the BRTI laboratory team (Tatenda Hamandawana, Simbarashe Maunganidze, Isabel Mashita, Agness Nhidza Manjoro, Justin Mayini) and Professors John Z. Metcalfe, Lovemore Gwanzura, Exnavier Gomo and Anthony Butterworth for their support and assistance. We also want to thank the National Microbiology Reference Laboratory (NMRL) where the BRTI TB Lab is housed.

This work was supported through capacity building grants awarded to BRTI from EDCTP (Trials of Excellence in Southern Africa), National Institutes of Health (International, Clinical, Operational and Health Services Research Training Award), Dr. JZ Metcalfe grant number: K23 AI094251) and Robert Wood Johnson Foundation AMFDP Medical Faculty Development Award.

Authors' contributions

RM designed the study, BM analysed the specimens, collected the data and wrote the 1st draft. SM collected the samples, TB analysed the data, VR supervised the study. All other authors contributed to the writing of the manuscript.

References

1. Batz HG, Cooke GS, Reid SD (2011) TB report - Towards lab-free tuberculosis diagnosis A strategic vision for R&D into point-of-care testing in resource-poor settings. Available: https://www.msfaccess.org/sites/default/files/MSF_assets/TB/Docs/TB_ReportSummary_TowardsLabFreeTBDX_ENG_2011.pdf. Accessed 14 November 2015.
2. Comolet T (2015) Multidrug-resistant tuberculosis: challenges of a global emergence. Bull Soc Pathol Exot 108: 290–298.
3. Zignol M, Gemert W.V, Falzon D, Sismanidis C, Glaziou, Floyd K, Raviglione M (2012) Surveillance of anti-tuberculosis drug resistance in the world: an updated analysis, Bull World Health Organ 90: 111–119.
4. IOM (Institute of Medicine) (2011) The Emerging Threat of Drug-Resistant Tuberculosis in Southern Africa: Global and Local Challenges and Solutions: Summary of a Joint Workshop. Washington DC National Academics Press.
5. Metcalfe JZ, Makumbirofa S, Makamure B, Sandy C, Bara W, Mungofa S, Hopewell PC, Mason P (2014) Drug-resistant tuberculosis in high-risk groups, Zimbabwe. Emerg Infect Dis 20: 135–137.
6. Tenover FC, Crawford JT, Huebner RE, Geiter LR, Horseburg CR, Good RC (1993) The resurgence of tuberculosis: is your laboratory ready? J Clin Microbiol 31: 767–770.
7. World Health Organization (2010) Launch of the WHO Global Tuberculosis Control Report 2010. Tuberculosis - World Health Organization.Available:http://www.who.int/tb/features_archive/global_report2010_launch_11nov10/en/. Accessed 14 November 2015.
8. Dara M, Kluga H (2011) Roadmap to prevent and combat drug-resistant tuberculosis. The Consolidated Action Plan to Prevent and Combat Multidrug- and Extensively Dru**g-**Resistant Tuberculosis in the WHO European Region 2011–2015 Available: http://www.euro.who.int/en/publications/abstracts/roadmap-to-prevent-and- combat-drug-resistant-tuberculosis. Accessed 13 November 2015.
9. Boehme C, Nabeta P, Hillemann D, Nicol MP, Shenai S, Krapp F, Allen J, Tahirli R, Blakemore R, Rustomjee R, Milovic A, Jones M, O'Brien SM, Persing David H, Ruesch-Gerdes S, Gotuzzo E, Rodrigues C, Alland D, Perkins MD (2010) Rapid molecular detection of tuberculosis and rifampin resistance. N Engl J Med 363: 1005–1015.
10. Shah N.S, Moodley P, Babaria P, Moodley S, Ramtahal M, Richardson J, Heysell S, Li X, Moll A, Friedland G, Sturm AW, Gandhi NR (2011) Rapid diagnosis of tuberculosis and multidrug resistance by the microscopic-observation drug-susceptibility assay. Am J Respir Crit Care Med 183: 1427–1433.
11. Lee A, Teo A, Wong S (2001) Novel mutations in isoniazid-resistant *Mycobacterium tuberculosis* isolates. _Antimicrob. Agents Chemother 45: 2157–2159.
12. Marttila HJ, Soini H, Eerola E, Vyshnevskaya E, Vyshnevskiy BI, Otten TF, Vasilyef AV, Viljanen MK (1998) Thr substitution in KatG is predominant in genetically heterogeneous multidrug-resistant *Mycobacterium tuberculosis* isolates originating from the St. Petersburg area in Russia. Antimicrob. Agents Chemother 42: 2443–2445.
13. Chihota VN, Grant AD, Fielding K, Ndibongo B, Zyl AV, Muirhead D, Churchyard GJ (2010) Liquid vs. solid culture for tuberculosis: performance and cost in a resource-constrained setting. Int J Tuberc Lung Dis 14: 1024–1031.

14. Piatek AS, Tyagi S, Pol AC, Telenti A, Miller LP, Kramer FR, Alland D (1998) Molecular beacon sequence analysis for detecting drug resistance in *Mycobacterium tuberculosis*. Nat Biotechnol 16: 359–363.
15. Abimbola TO Marston BJ, Date AA, Blandford JM, Sangrujee N, Wiktor SZ (2012) Cost-effectiveness of tuberculosis diagnostic strategies to reduce early mortality among persons with advanced HIV infection initiating antiretrovi. J Acquir Immune Defic Synd 60: 1–7.
16. Aung MN, Moolphate S, Paudel D, Jayathunge Ph M, Duangrithi D, Wangdi K, Aung TN, Lorga T, Higuchi K (2013) Global evidence directing regional preventive strategies in Southeast Asia for fighting TB/HIV. J Infect Dev Ctries 7: 191–202. doi: 10.3855/jidc.2903.
17. Zeman DH (1997) The 'best' diagnostic test. J Swine Health Prod 4: 159 – 160.
18. Chen YS, Liu PY, Huang YF, Chen CS, Chiu LH, Huang NY, Hsieh KS (2013) Comparison of diagnostic tools with multiplex polymerase chain reaction for pediatric lower respiratory tract infection: A single center study. J Microbiol Immunol Infect 46: 413–418.
19. Piatek AS, Van Cleeff M, Alexander H, Coggin WL, Rehr M, Kampen SV, Shinnick TM, Mukadi Y (2013) GeneXpert for TB diagnosis: planned and purposeful implementation. Glob Health Sci Pract 1: 18–23.
20. Aber VR, Allen BW, Mitchison DA, Ayuma P, Edwards EA, Keyes AB (1980) Quality control in tuberculosis bacteriology. 1. Laboratory studies on isolated positive cultures and the efficiency of direct smear examination. Tubercle 61: 123–133.
21. Colebunders R, Bastian L (2000) A review of the diagnosis and treatment of smear-negative pulmonary tuberculosis. Int J Tuberc Lung Dis 4: 97–107.
22. Lawn SD, Nicol PM (2011) Xpert MTB/RIF assay: development, evaluation and implementation of a new rapid molecular diagnostic for tuberculosis and rifampicin resistance. Future Microbiol 6: 1067–1082.
23. Guillerm M, Usdin M, Arkinstall J (2006) TB Diagnostics and Drug Sensitivity Testing An overview of the current diagnostic pipeline. Paris. Available: https://www.doctorswithoutborders.org/sites/usa/files/tb_diagnosis_oct2006.pdf. Accessed 6 June 2016
24. Mueller DH, Mwenge L, Muyoyeta M, Tembwe R, Godfrey-Faussett P, Ayles HM (2008) Costs and cost-effectiveness of tuberculosis cultures using solid and liquid media in a developing country. Int J Tuberc Lung Dis. 12: 1196–1202.
25. World Health Organization Policy Statement (2011) Noncommercial culture and drug-susceptibility testing methods for screening patients at risk for multidrug resistant tuberculosis.Available:http://apps.who.int/iris/bitstream/10665/44601/1/9789241501620_eng.pdf Accessed 14 November 2015.
26. Solari L, Gutierrez A, Suarez C, Jave O, Castillo E, Yale G, Ascencios L, Quispe N, Valencia E, Suarez V (2011) Cost analysis of rapid methods for diagnosis of multidrug resistant tuberculosis in different epidemiologic groups in Perú. Rev Peru Med Exp Salud Publica 28: 426–431.

Corresponding author

Beauty Makamure
Laboratory Manager
Medical Microbiology,
Biomedical Research and Training Institute,
10 Seagrave Road,
Cnr S.Nujoma St and Seagrave road,
P.O.Box CY 1753, Cuaseway,
Harare, Zimbabwe
Phone: 263 4 336691
Mobile: 263 774 168138
Fax: 263 4 333 464
Email: makamureb@gmail.com

Conflict of interests: No conflict of interests is declared.