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ORIGINAL RESEARCH

The study to compare XPERT MTB/RIF with line probe assay for detection of rifampin-monoresistant mycobacterium tuberculosis

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ABSTRACT
TB is the leading cause of death from a single infectious agent in the world. The advent of MDR-TB has further complicated the situation. The use of culture-based drug resistance testing is hindered by unacceptable long turn around for results and high contamination rates. The technological difficulties of conducting a TB culture test causes a problem for case diagnosis and initiation of treatment.This is a prospective cross sectional, descriptive study conducted among the sputum positive presumptive DR-TB patients.The study was conducted over a period of one year. The sensitivity and specificity of GeneXpert MTB/RIF for the detection of Rifampicin mono resistance in our study was 60% and 94% and for LPA it was found to be 100% and 100% respectively. Further analysis of samples by MGIT-DST showed discrepancy between LPA and GeneXpert results, confirms 100% agreement between MGIT 960 and LPA. LPA has a better efficiency characteristic than GeneXpert and an alternative to culture for the diagnosis of RIF mono-resistance.

Key words: GeneXpert, Lpa, MGIT-DST,Rifampicin monoresistance

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by the bacillus 'Mycobacterium tuberculosis'. The most common site of infection is the lungs (pulmonary TB), but can also affect other sites (extra-pulmonary TB). Worldwide, TB is one of the top 10 causes of death, and the leading cause from a single infectious agent (above HIV/AIDS). About a quarter of the world's population is infected withM. tuberculosis and thus at risk of developing TB disease.

Globally in 2018, an estimated 10.0 million (range, 9.0-11.1 million) people fell ill with TB, equivalent to 132 cases (range, 118-146) per 100 000 population. There were an estimated 1.2 million (range, 1.1-1.3 million) deaths from TB among HIV-negative people in 2018 and an additional 251 000 (range, 223 000-281 000) deaths from TB among HIV-positive people (33% of the total number of deaths caused by HIV/AIDS)[1].In 2019, an estimated 3.3% of new cases globally and 18% of previously treated cases had MDR/RR-TB. Overall, there were an estimated 465 000 MDR/RR-TB incident cases in 2019 (10% increase from 2018) and the global proportion of cases of RR-TB estimated to have MDR-TB was 78%[2].

The emergence of multidrug and extensively drug-resistant tuberculosis (MDR-TB and XDR-TB, respectively) is a major threat to global tuberculosis control[3].Rifampicin-resistant TB strains may be susceptible or resistant to isoniazid (i.e. MDR-TB), or resistant to other first-line TB medicines (polyresistant) or second-line TB medicines (e.g. extensively drug-resistant [XDR]-TB)[4].Smear microscopy is the mainstay for the diagnosis of TB in resource-limited settings but it has low (35-80%)sensitivityandapoorpositivepredictivevalue(PPV) [5]. Culture and drug-susceptibility testing (DST) using solid media may take up to 8-12 weeks for the results[6] and faster liquid-based culture methods takes 4-6 weeks[7].The delay associated with DST lead to prolonged periods of ineffective therapy and ongoing tuberculosis transmission. Hence, there is need of

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introduction of new rapid diagnostic tools to detect DR-TB[8].A new rapid molecular test that was recommended for use by WHO in December 2010, the Xpert MTB/RIF assay is an automated, real- time nucleic acid amplification technology (Catridge based NAAT)[9].The Xpert MTB/RIF assay simultaneously detects Mycobacterium tuberculosis and rifampicin resistance causing mutations in a closed system suitable for use outside conventional laboratory settings in less than 2 hours, directly from sputum samples[10].Line probe assay (LPA) is based on polymerase chain reaction and it detects MTB complex as well as drug sensitivity to rifampicin and isoniazid. In both of this rapid tests, mutations in the 81-bp hotspot region of the rpoB gene, helps in the detection of rifampicin resistance[11].Both of the molecular technologies are well established for rapid diagnosis and RIF-resistance detection in *M.tuberculosis*, but a systematic comparison of these two tests with standard liquid culture (MGIT960)-based DST is rarely done. This study is to compare the efficacy and accuracy of the Xpert MTB/RIF and LPA in cases of RIF monoresistance compared to the gold standard MGIT960 culture-based DST.

METHODOLOGY

SOURCE OF DATA

This study was conducted among the sputum positive presumptive DR-TB patients visiting Hospital.

METHOD OF COLLECTION OF DATA

STUDY DESIGN: Prospective cross sectional, descriptive study.

SAMPLE SIZE CALCULATION

The study group consists of subjects more than or equal to 18 years of age and will be included in study after taking informed consent.

Sample size will be calculated based on following formula-

$$\text{Sample size} = \frac{Z\alpha^2\ P(1-P)}{d^2}$$

Za2 = Standard normal variate at 5% = 1.96
P = Prevalence of MDR = 10.9%
d = Absolute error.

Sample size required = 236 (5% error)

Based on inclusion and Exclusion criteria a minimum of 250 sputum positive presumptive drug-resistant Tuberculosis patients will be selected.

INCLUSION CRITERIA

- All sputum positive presumptive Drug Resistant-Tuberculosis patient including Relapse, Recurrent, Losed to follow-up, Retreatment cases, Treatment failure, Contacts of MDR-TB.

EXCLUSION CRITERIA

Cases less than 18 years of age.

Sputum sample from presumptive DR-TB cases are collected in sterile Falcon tube after thorough rinsing of oral cavity with clean water. Sputum sample along with form containing details of patient like Name, Age, Sex, Address, Type of family, HIV status and name of hospital are documented and sent to NTEP lab. Samples were used to perform AFB, CBNAAT, FL-LPA. CULTURE/DST is performed in patients whose sputum test results showed discrepancy in CBNAAT and LPA.

RESULTS

Table 1: LPA results

LPA results	Frequency	Percent
Invalid	2	0.8%
MDR (INHr/RIFr)	30	12%
INH Monoresistance (INHr)	60	24%
Sensitive to both (INHs/RIFs)	95	38.3%
RIF Monoresistance (RIFr)	63	27.4%

Out of 250 smear-positive presumptive DR-TB samples, 2 (0.8%) showed invalid results on the LPA. Of the remaining 248 samples, 95 (38.3%) were susceptible to both INH and RIF, 60 (24%) had MDR, 30 (12%) showed INH monoresistance, and 63 (27.4%) showed RIF monoresistance. 158 samples which includes RIF-monoresistant (63 cases) and 95 drug susceptible cases were further analyzed by Xpert MTB/RIF.

Overall, 158 samples were tested for GeneXpert MTB/RIF. (63 RIF-monoresistant and 95 drug susceptible cases by LPA).

Out of these 63 RIF-monoresistant samples on GeneXpert testing, 38 (60.31%) showed RIF-resistant M. tuberculosis and 25 (39.6%) were found to have RIF-susceptible *M. tuberculosis* (Graph 8).

The 95 samples that were drug susceptible by LPA, all were subjected to the Xpert MTB/RIF assay. Of these, 2 (2.1%) samples showed errors, 88 (92.6%) found to be drug susceptible, and 5 (5.2%) were RIF resistant samples. (Graph 8).

Thus, the overall concordances between the LPA and Xpert MTB/RIF were 60.31% (n=38) and 92.6% (n=88) for the detection of RIF-resistant and RIF-susceptible strains respectively.

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There were 25 LPA RIFr/Genexpert RIFs and 5 LPA RIFs/GeneXpertRIFr samples which shows discrepancy between LPA and Xpert MTB/RIF.

30 LPA and Xpert MTB/RIF discrepant samples were tested by the MGIT960 culture DST method as the gold standard. Of these, 25 were LPA RIFr/Xpert MTB/RIFs and 5 were LPA RIFs/ Xpert MTB/RIFr. In MGIT960, one culture from each group got contaminated.

Of the remaining 28 M.tuberculosis isolates, all 24 (96%) LPA RIFr/GeneXpert RIFs samples gave Rifampicin resistance and all 4 (80%) LPA RIFs/GeneXpertRIFr were rifampicin sensitive.This shows MGIT results are in concordance with LPA results but high discordance with Xpert MTB/RIF result.

Out of 63 LPA RIFr samples, 38 (60.31%) had RIF-resistant *M. tuberculosis* (GeneXpertRIFr) and 25 (39.6%) were found to have RIF-susceptible *M. Tuberculosis* (GeneXpert RIFs). (Graph 8)

25 LPA RIFr/GeneXpert RIFs samples were subjected to MGIT culture/DST,1 sample got contaminated, remaining 24 (96%) samples confirms Rifampicin Monoresistance. (Graph 8)

Out of 95 LPA RIFs/INHs samples, 2 (2.1%) gave indeterminate result, 88 (92.6%) showed RIF-sensitive *M. tuberculosis* (GeneXpert RIFs) and 5 (5.2%) were found to have RIF-resistant *M. Tuberculosis*(GeneXpertRIFr).

5 LPA RIFs/GeneXpertRIFr samples were subjected to MGIT culture/DST, 1 sample got contaminated, remaining 4 (80%) samples showed susceptible to rifampicin.

Table 2: Comparison of LPA and GeneXpert report

LPA (n-158)	Xpert MTB/RIF (n-158)			
	Resistant	**Sensitive**	**Error**	**Total**
LPA RIFR	38	25	0	63
LPA RIFS	5	88	2	95
Total	**43**	**113**	**2**	**158**
Sensitivity-60.32% (CI-47.20% to 72.43%)				
Specificity-94.62% (CI-87.90% to 98.23%)				

Of 158 samples which GeneXpert was carried out (including 63 RIF monoresistance and 95 drug sensitive cases) 38 samples were LPA RIFr/GeneXpertRIFr, 25 were LPA RIFr/GeneXpert RIFs, 5 samples were LPA RIFs/GeneXpertRIFr and the remaining 88 were LPA RIFs/GeneXpert RIFs.

For the detection of Rifampicin mono resistance, the GeneXpert MTB/RIF sensitivity and specificity were 60.32% (CI-47.20% to 72.43%) and 94.62% (CI-87.90% to 98.23%).

Table 3: Comparison of LPA with DST report

LPA (n-30)	MGIT-DST (n-30)			
	Resistant	**Sensitive**	**Error**	**Total**
LPA RIFR	24	0	1	25
LPA RIFS	0	4	1	5
Total	**24**	**4**	**2**	**30**
Sensitivity-100% (CI-85.75% to 100.00%)				
Specificity-100% (CI-39.76% to 100.00%)				

Of 30 samples which showed discrepancy with LPA and GeneXpert (including 25 LPA RIFr/GeneXpert RIFs and 5 LPA RIFs/GeneXpertRIFr) on MGIT/DST, 24 samples were LPA RIFr/MGIT RIFr, 4 were LPA RIFs/GeneXpert RIFs, 2 samples showed error.

For the detection of Rifampicin mono resistance, the sensitivity and specificity of LPA is 100% (CI-85.75% to 100.00%) and 100% (CI-39.76% to 100.00%) respectively.

DISCUSSION

Sensitivity of the GeneXpert assay to detect resistance to Rifampin has been reported to be between 60% to nearly 100%, depending on the characteristics of the tested population and bacterial loads in the sample[11].The sensitivity and specificity of GeneXpert MTB/RIF for the detection of Rifampicin mono resistance in our study was 60% (CI-47.20% to 72.43%) and 94% (CI-87.90% to 98.23%).

whereas the specificity matched with similar research for example, a study in South Africa, recorded 94% specificity for GeneXpert MTB/RIF. Similarly, in an Asian group, there was a pooled specificity of between 68% -100%. It was noteworthy that our study documented lower sensitivity. Previous studies have suggested explanations for this, such as the need for new specific probes in various regions of identification of mutations in GeneXpert[12].

The sensitivity and specificity of rifampicin mono resistance of LPA found in this research was 100% (CI-85.75% to 100.00%) and 100% (CI-39.76% to

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100.00%) respectively which was consistent with other studies. For instance, the sensitivity and specificity of both the South African and South American population was 92% and 97% respectively. Equally, a sensitivity of 96 percent was reported working with high-risk MDR-TB set up in Taiwan. In comparison, a study in New Delhi reported a sensitivity and specificity of 97.6% and 94.4 %similarly study in South Africa documented sensitivity and specificity of 97.7% and 91.8% respectively the sensitivity and specificity of the detection of Rifampicin mono resistance was 96.4% and 100% among smear positive samples in Ethiopia and 100% and 96.1% among the smear positive population in Uganda[13].

Similarly by Rufai *et al*., (134) in 2014 reported 100% agreement between MGIT 960 and LPA results and Yadav *et al*., in 2013 by reporting the same with the conventional DST at 96% in New Delhi.

Xpert MTB/RIF become so popular because, it has shorter processing time and can also detect rifampicin resistance. However, after its extensive use and review, reports have started to show that it can yield false-negative and false-positive RIF resistance results[14].

Our analysis reveals that only **60.31% (n=38)** of cases of RIF monoresistant TB were correctly diagnosed with Xpert MTB/RIF. The remaining **39.68% (n=25)** of cases were found to be falsely RIF susceptible. With mixed MTBC infections, the Xpert MTB/RIF assay has a increased false-negative rate for detecting rifampin resistance especially in poor outcome setting like drug defaulter, retreatment, immunocompromised states and might require further clarification[15].

Liquid-based mycobacterial culture is challenged by contamination rates that can be as high as 14.0% to 18.6% when comparing line probe assay. In our study Invalid rates of the line probe assay were lower (**0.8%**), than the MGIT contamination rate which is **6%.**

MTB isolates are mistakenly categorised as susceptible, causing underestimation of the incidence of MDR-TB. False-negative reports of RIF resistance will keep patients on first-line medications unnecessary for a long time, leaving patients inappropriately untreated. This can contribute to the amplification and spreading of MDR and XDR-TB [16].

CONCLUSION

For detecting Rifampicin mono-resistance, Gene Xpert MTB/RIF had sensitivity, specificity lower than LPA, and the MGIT960 results showed 100% agreement with LPA results. LPA has a better efficiency characteristic than GeneXpert and an alternative to culture for the diagnosis of RIF mono-resistance.

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