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Original Research Article

A retrospective analysis of drug resistance in *M. tuberculosis* and role of CBNAAT, LPA and culture in diagnosis

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ABSTRACT

Background: Nucleic acid detection has potentially revolutionized diagnosis of tuberculosis and has established as a screening test of choice. However, conclusions on its role in diagnosing extrapulmonary infection and discordance between drug susceptibility reported through culture, Xpert MTB/ RIF, line probe assay require further review. Objectives were to compare positivity rate of Xpert MTB/RIF ULTRA across various sample types; compare drug susceptibility percentage of *Mycobacterium tuberculosis* (M. tb) across three platforms i.e., culture, Xpert MTB/RIF and LPA

Methods: A retrospective analysis of results of samples was undertaken for a period of one year for Xpert MTB/RIF ultra and three years for LPA and susceptibility through MGIT.

Results: Xpert MTB/ RIF Ultra showed overall positivity of 26%, with 10% rifampicin resistance; genitourinary sample positivity was 4%. First line LPA recorded 26% Rif resistance and very few Rifampicin indeterminates. Second line LPA revealed 5.4% aminoglycoside resistance and 26% fluoroquinolone resistance. Through MGIT Rif resistance was 18.2%, multidrug resistance 17.5%, isoniazid monoresistance 6.6%, FQ resistance 18.6%, MDR with FQ resistance 18.6%, amikacin resistance 4% and streptomycin resistance 18%.

Conclusions: Xpert MTB/ RIF should be used as a test of choice for detection; Rifampicin resistance should be confirmed with LPA. However, for GUN, pleural fluid and GIT tissue samples; an additional culture should be attempted on the primary sample to improve detection rates. Drug resistance detected through LPA should be phenotyped especially for fluoroquinolones. Moxifloxacin and amikacin could be empirical antibiotics of choice over ofloxacin and Kanamycin due to lower resistance percentage recorded for them.

Keywords: Xpert MTB/RIF, LPA, Culture

INTRODUCTION

Tuberculosis (TB) remains a global and a national challenge for India as per 'India TB report, 2022' in year 2020 the incidence of tuberculosis in India was 188 per 100000 population. A total of over 19 lakh old and new patients were notified and estimated mortality rate was 34-40 per 100000 population.[1] In addition a high burden of drug resistant tuberculosis was also reported. The introduction of molecular genotypic tests for the detection of M. tb and drug resistance by the WHO has been a useful addition. In March 2021, government of India released 'Guidelines for programmatic management of drug resistant tuberculosis in India' with aim of aligning it to world health organization (WHO) end TB strategy with recommendation to use cartridge based nucleic acid amplification test as the first line test for screening of patients for M. tb and detecting Rif

resistance, followed by line probe assay to confirm Rif resistance and to detect isoniazid (H) resistance if the screening test for mycobacterium is positive and to label the case as MDR and confirmation of susceptibility results by culture method. However, the uncertainty of using cartridge-based PCR tests i.e., Xpert MTB/ RIF for extrapulmonary samples, which are often paucibacillary, remains. There is a need to study the performance of Xpert MTB/RIF on extrapulmonary samples as it remains test of choice with clinical practitioners. Lower sensitivity of Xpert MTB/ RIF in detection of Rif resistance and confirmation of results of any molecular test, including LPA, with a phenotypic test is reported.[2,3] But the difference has not been extensively studied through data.

In addition, the requisitions for drug resistance testing which are received in a private laboratory is not as per WHO TB elimination guidelines and in most cases either an LPA or MGIT based sensitivity testing is opted. Thus, it is important to bring out the differences in the results (other than turnaround time) of these platforms for educational purposes.

Through this study we aim to evaluate percentage positivity of Xpert MTB/RIF Ultra for various samples and compare susceptibility results of antitubercular drugs obtained from LPA (FL and SL) and MGIT.

METHODS

A retrospective analysis for positivity rate across different sites and drug resistance percentage for various antibiotics was studied on results obtained from samples received at Metropolis healthcare limited, Mumbai from various parts of India.

Inclusion criteria

For analysis 33,052 results from Xpert MTB/ RIF Ultra, collected from a period of May 2021 to May 2022; 994 results on FL LPA and 518 results on SL LPA, collected over a 3-year period (2019 to 2022) and drug resistance profiles through MGIT for streptomycin, INH, Rif, Ethambutol (1844 samples each); Moxifloxacin, Clofazimine and Amikacin (642 samples each); ethionamide, para amino salicylic acid, ofloxacin (1269 samples each) and pyrazinamide (1147 samples each), collected over a 3-year period (2019 to 2022) were included.

Samples were tested on Xpert MTB/ RIF Ultra (Cepheid, USA), GenoType MTBDRplus VER 2.0 (Hain Lifescience, Germany), GenoType MTBDRsl VER 2.0 (Hain Lifescience, Germany) as per manufacturers recommendations. Drug susceptibility testing was done using MGIT medium on BACTEC 960. Critical concentration and method were as per 'Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis' by WHO, 2018. [4]

Exclusion criteria

Positivity rate at different body sites was studied for Xpert MTB/ RIF only; this was excluded for LPA and culture-based tests as requisitions for these tests were primarily received as an add-on-test post a positive-culture and re-booked in LIS as a fresh sample without actual sample site details. Due to the above reason a comparison of drug susceptibility results on the three platforms i.e., Xpert MTB/RIF, LPA and MGIT was also excluded.

RESULTS

Demographic data

A total of 33052 patients were tested for GeneXpert testing. Out of this 38.18% belonged to 18-45 years age group followed by 29.56% belonging to >60 years age group. Around 54.29% were males and 45.71% were females. The samples tested for pulmonary TB and extra pulmonary TB was somewhat similar (51.85% vs 48.15% respectively).

Table 1: Demographic analysis of samples tested by Xpert MTB/ RIF Ultra.

Variables	Frequency	Percentage (%)
Age group (Years)		
1-12	1372	4.15
13-18	1867	5.65
18-45	12619	38.18
46-60	7423	22.46
>60	9771	29.56
Gender		
Female	15108	45.71
Male	17944	54.29
Tested for		
Extra pulmonary Tb	15916	48.15
Pulmonary Tb	17136	51.85

Xpert MTB/ RIF ultra

A total of 33052 samples were tested out of which 15, 183 (46%) were pulmonary samples and remaining extrapulmonary. Percentage positivity and rifampicin results were tabulated basis site of sample collection (Table 2).

Overall, M. tb was detected in 8741 (26%) of the samples tested. Rif resistance was detected in 10% of the positive samples whereas 15% Rif results were indeterminate.

First line LPA

The 994 samples tested for first line LPA, included 549 (55%) pulmonary samples and 445 from extrapulmonary sites. For site-specific Rifampicin resistance refer to Table 3.

The overall rifampicin resistance was 26% There were 04 cases with indeterminate results. The overall isoniazid resistance (not shown in table) was 34% while monoresistance was 10.6%. Isolated INHA mutation was observed in one out of total 321 cases.

Second line LPA was performed on 518 M. tb culture isolates. Aminoglycoside resistance was detected in 5.4% and fluoroquinolone (FQ) resistance in 26%. Indeterminate results were observed in 9 cases (Table 4).

MGIT based drug susceptibility testing

The 1884 M. tb isolates were tested for various antibiotic combinations as per treating doctor's requirements. Resistance to Rif, INH and MDR were detected in 18.2%, 25.1% and 17.5% of isolates. Overall fluoroquinolone resistance was 18.6% and MDR with fluoroquinolone resistance (both moxifloxacin and ofloxacin) was 18.6%. Resistance to any one of aminoglycosides i.e., amikacin, kanamycin or capreomycin was 4.9% (Table 4).

Table 2: Site specific percentage positivity and Rif resistance by Xpert MTB/ RIF Ultra.

Sample type	Grand total	Detected	Not detected	% positive	% Rif resistant	% Rif indeterminate	% Rif sensitive
Respiratory samples	15183	4866	10317	32	11	12	77
Pleural fluid	3719	450	3269	12	4	51	45
Tissue	1965	497	1468	25	9	23	68
Tissue (only GIT)	337	57	280	17	9	53	53
L. node	1057	483	574	46	10	19	71
Genitourinary	685	30	655	4	3	40	57
Abscess	583	196	387	34	12	9	79
Spinal tissue and abscess	120	48	72	40	10	15	75
Bone	125	38	87	30	11	21	68
Others	9278	2076	7202	22	10	12	79
Grand total	33052	8741	24311	26	10	15	75

Table 3: Site specific Rif resistance by FL LPA.

Sample site	Grand total	Inconclusive	Indeterminate	Resistant	Resistant inferred	Susceptible	% Res
Respiratory samples	549	1	2	151	1	394	28
Pleural fluid	16		1	5		10	31
Tissue	31		1	4		26	13
L. node	32			9		23	28
Abscess	101			29		72	29
Others	265			59		206	22
Grand total	**994**	1	4	257	1	731	26

Table 4: Comparison of percentage resistance detected by Xpert MTB/RIF Ultra, FL LPA, SL LPA, MGIT (DST).

Antibiotics	Xpert MTB/ RIF ultra, no. tested (%)	FL LPA, no. tested (%)	SL LPA, no. tested (%)	DST, no. tested (%)
Rifampicin resistance	33052 (10.2)	994 (25.8)	-	1844 (18.2)
MDR	-	994 (22.4)	-	1844 (17.5)
INH mono resistance	-	994 (10.6)	-	1844 (6.6)
Rifampicin monoresistance	-	994 (3.4)	-	1844 (0.2)
FQ (any)	-	-	518 (26.1)	1239 (18.6)
Kanamycin/ amikacin/ capreomycin (Any)	-	-	518 (5.4)	1239 (4.9)
MDR with FQ resistance (both moxifloxacin, ofloxacin)	-	-	-	322 (18.6)
MDR with amikacin resistance	-	-	-	322 (4.0)
Both aminoglycosides and quinolone any	-	-	518 (4.4)	-
Isoniazid	-	-	-	1844 (25.1)
Pyrazinamide	-	-	-	1147 (26.7)

Continued.

Antibiotics	Xpert MTB/ RIF ultra, no. tested (%)	FL LPA, no. tested (%)	SL LPA, no. tested (%)	DST, no. tested (%)
Ethambutol	-	-	-	1874 (7.2)
Amikacin	-	-	-	642 (4.0)
Streptomycin	-	-	-	1874 (18)
Moxifloxacin	-	-	-	642 (12)
Ofloxacin	-	-	-	1269 (18.3)
Clofazimine	-	-	-	642 (0.5)
Capreomycin	-	-	-	1147 (2.8)

DISCUSSION

Early diagnosis of tuberculosis and detection of drug resistance is crucial for successful treatment and controlling spread of the disease. Genotypic tests i.e., cartridge-based nucleic acid amplification assay and LPA have substantially reduced the turnaround time. However, for susceptibility testing phenotypic methods are still considered gold standard against which other methods are compared as they can help identify bacterium which is not expressing the mutation that had been detected there by allowing de-escalation.

Xpert MTB/RIF, popular due to its ease of performance and adoption by WHO as the screening test of choice for respiratory and few extrapulmonary samples has a limitation in terms of variety of samples that can be tested. In a metanalysis published by WHO the median (%) pooled sensitivity for lymph nodes, CSF, pleural fluid, gastric lavage, and other tissues samples, when compared against culture, was 84.9%, 79.5%, 43.7%, 83.8% and 81.2%.[5]

In this study we found high percentage of detection in the following extrapulmonary samples: lymph node, spinal tissue, bone and abscess and a low positivity in pleural fluid, GUN, and GIT tissue. In addition, the low positivity samples showed higher Rif indeterminate results thus further obscuring use of Xpert MTB/RIF ULTRA in these samples. This is similar to low-positivity percentage obtained by Kashyap et al in GUN; and high-positivity percentage obtained by Agarwala et al in-liver abscess; and low-positivity percentage, in pleural fluid, and high-positivity percentage in lymph nodes and abscess by Chaudhary et al.[6-8]

False positive and false negative calls by Xpert MTB/ RIF have been reported by authors earlier.[2,3] Xpert MTB/RIF ULTRA with better chamber dynamics and melting temperature-based analysis has been stated superior to its previous version for sensitivity of detection of M. tb and Rif resistance; in this study group Rif resistance was detected in only in 10% cases by Xpert MTB/RIF ULTRA as compared to 25.8% by LPA and 18.2% by liquid culture which shows that still a significant difference exists between ability of Xpert MTB/RIF ULTRA and LPA to detect Rif resistance and thus one must follow a M. tb detected by Xpert MTB/ RIF with LPA as suggested in WHO guidelines.[9,10]

Between LPA with MGIT a significant difference in detection of resistance for fluoroquinolones was noted (26.1% vs 18.6%) in comparison to aminoglycosides (5.4% vs 4.9%). Percentage resistance difference was comparable to the differences observed by Maningi et al i.e., 7.7% and 1.2% for ofloxacin and kanamycin respectively.[11] Ideally all molecular results should be confirmed with phenotypic but if cost constraints do not permit than phenotypic confirmation of fluroquinolones should be a priority over aminoglycosides.

This study showed that percentage drug resistance for Rif, streptomycin was similar to resistance % published by Lohiya et al which had included a total of 90 non-duplicate studies published till 2018 for their review.[12]

An 18% resistance to streptomycin and Rif noted in our study (we did not differentiate between new and previously treated cases due to lack of history in many cases) was comparable to the average of resistance % observed in meta-review by Lohiya et al wherein they had documented a resistance of 20% (average of new and previously treated cases).[12]

However, the increase in resistance to pyrazinamide was observed (current: 26.7%; Lohiya et al 15%).[12]

Our data showed a low resistance to aminoglycosides 5.4% and 4.9% by LPA and MGIT (refer table) thus upholding their use potential if need arises. Overall Moxifloxacin and Amikacin were the antibiotics of choice among their class due to lower resistance percentage.

The major limitation in any retrospective study where in a patient undergoes multiple tests during course of treatment is traceability. Our chain of laboratories is now working on unique patient identification number linked to mobile so that results of tests undertaken at different time intervals can be studied together.

CONCLUSION

Xpert MTB/ RIF should only be used as a screening test for M. tb detection; Rif resistance should be confirmed with LPA before starting treatment. Xpert MTB/RIF should not be used as a stand-alone test for detection of tuberculosis in GUN, pleural fluid and GIT tissue samples; in these cases, additionally a culture should be

attempted on the primary sample to improve detection rates. Drug resistance detected through LPA should be confirmed with manual method to confirm phenotype; if cost is a constraint, then confirmation should at least be sought for quinolones. Moxifloxacin and amikacin could be empirical antibiotics of choice over ofloxacin and Kanamycin due to lower resistance percentage recorded for them.

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