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Impact of Gene Xpert in Resource Limited Settings to Meet End TB Strategy

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[1]Main concept, [2]Main concept and writing the manuscript, [3] Lab work and compilation of data, [4,5]Calculation of results and writing, Compilation of data and calculation of results, [6]Calculation of results

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ABSTRACT

Objective: To extensively analyze the diagnostic accuracy of Gene Xpert MTB/RIF assay in cases of both pulmonary and extra pulmonary TB, versus the standard conventional methods like Ziehl Neelsen smear microscopy.

Methodology: This study was conducted from August, 2023 to March 2024 at Microbiology Laboratory MTI- GKMC/BKMC, Swabi. Samples received both from pulmonary and/or extra-pulmonary sites, were subjected to formation of smear followed by microscopy on Ziehl Neelsen staining, and further subjection to Gene Xpert analysis.

Results: Out of the total 863 samples, 78 were detected positive on Gene Xpert, with an overall positivity of 9.03%, whereas the overall positivity on microscopy was lower i.e. 8.45%. Overall Rifampicin resistance as seen on Gene Xpert analysis was 1.28%, as only one pus sample out of a total of 78 pulmonary and extra pulmonary samples, was detected to be Rifampicin resistant. Diagnostic performance of Gene Xpert MTB/RIF in comparison with Microscopy was calculated using the following 2X2 table, taking Gene Xpert as a standard which showed a sensitivity of 93.58% and specificity of 100%, a positive predictive value was calculated to be 100%, a negative predictive value of 99.36% with an accuracy of 99.42%.

Conclusion: Gene Xpert MTB/RIF assay has proven to be a highly sensitive user-friendly technique for diagnosing TB and resistance against rifampicin simultaneously for both pulmonary and extra pulmonary TB.

Key words: Gene Xpert MTB/RIF assay, MDR-TB, Mycobacterium tuberculosis, Rifampicin.

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Introduction

Apart from more than 500,000 per annum cases of TB being reported, even worse is that more than 15000 drug resistant TB cases are reported from Pakistan every year.[1]

Pakistan was ranked to have fourth highest prevalence of multidrug-resistant TB (MDR-TB) worldwide and was ranked fifth among the countries with highest burden globally as it accounts for 61% of the TB burden in the WHO, Eastern Mediterranean Region.[1]

Tuberculosis, a deadly infectious disease predominantly affecting the lungs, but also capable of disseminating to multiple organs within the body including the central nervous system, cardiovascular system, gastrointestinal tract and genitourinary tract etc.[2] thus divided into Pulmonary and Extra pulmonary TB.[3] Usually a disease of the immunocompromised and also reported in children, extra pulmonary TB rarely has a positive sputum culture[2,3] thus making it quite challenging to diagnose not only for the microbiologists, but for the physician as well.[4] and the rate of drug resistance reported is even higher in extra pulmonary type of TB.[5]

Delayed diagnosis and poor compliance both in terms of following the treatment regimens, along with poor quality medications are the major factors responsible for the emergence of increased number of drug resistant cases of tuberculosis.[1]

Multidrug resistant organism is the one resistant to at least two main first line anti tuberculous drugs i.e. Rifampicin and Isoniazid. The route of becoming infected with MDR-TB would either be by exposure to an already resistant strain of Mycobacterium or due to lack of compliance to treatment leading to the selection of a resistant strain.[6]

Extensively drug-resistant TB abbreviated as XDR-TB is a form of the already existing MDR-TB which develops resistance to more anti tuberculous drugs and thus further reducing the treatment options, which has already been declared by WHO in 117 countries globally.[7]

According to the Sustainable Development Goals (SDGs), which aim to transform our world, the target to be achieved by 2030 is to "end the epidemics of AIDS, TB, malaria and neglected tropical diseases, and combat hepatitis, water-borne diseases and other communicable diseases".[8]

The achievement of this goal globally requires rapid and accurate diagnosis followed by prompt treatment. Although microscopy with ZN staining for acid fast bacilli is the most frequently used technique in the Microbiology for diagnosing Mycobacterium tuberculosis, the associated limitations often lead to misdiagnosis.[9]

No doubt in culturing mycobacterium, being the gold standard for diagnosis of TB, but it definitely is time consuming and therefore the patients till they get their results are a constant source of transmitting the disease to others and it might be late enough that the patient may actually not be there to receive the result.[10]

Recommended in the year 2010, by the World Health Organization, Xpert® MTB/RIF (Cepheid, Sunnyvale, United States of America [USA]), [12] is a fully automated real-time semi-nested PCR assay for the detection of TB and rifampicin-resistant TB simultaneously.[12]

Methodology

After taking the ethical approval from the institutional review board 863 samples were collected from patients at Microbiology Department, coming to OPD or admitted patients in Bacha Khan Medical Complex, Swabi in the said time period.

Inclusion criteria: Both the out patients and in patients of all genders and ages with suspected pulmonary/extra pulmonary TB were included.

Exclusion criteria: Repeated samples from the same patients were excluded from the study. Patient's demographic data was also recorded.

Samples included were mainly sputum samples i.e 751 out of a total of 863 samples received. A total of 80 fluids including ascitic fluid (42) and pleural fluid (36) BAL (02). For patients who were unable to expectorate the sputum, ultrasonic nebulizer technique was used for sputum induction. The remaining samples included CSF (13), Pus (11), Stool (07), and Urine (01).

After preparing smears from all the samples, Ziehl-Neelsen staining was performed on those smears. Thorough examination of the smears was carried out to look for acid-fast bacilli and graded as per the International Union against Tuberculosis and Lung Disease scale; negative for TB, scanty, + 1, +2, and + 3. A patient was considered positive if a minimum of one smear was graded scanty or higher.[13]

As per the provider information and guidelines for interpretation, "It is a point of care, nucleic acid amplification method that has been designed as an integrated DNA extraction and real time PCR system. A reagent is added to a concentrated sputum pellet to liquefy the specimen and kill any mycobacteria within. The cartridge is loaded into the Gene Xpert Analyzer, which subsequently detects the presence of TB DNA and any mutations that confer rifampin resistance. Internal controls are present within each cartridge to assess the presence of inhibitors that may cause false negative results".[9]

Results

Out of the total 863 samples, 78 were detected positive on Gene Xpert, with a positivity of 9.03%,, which was lower than the overall positivity received on microscopy i.e. 8.45%.

71(91.02%) of the total positive samples were pulmonary in origin, whereas the remaining 07 (8.97%) were extra pulmonary in origin.

Regarding the demographic details of the patients included in the study as shown in Table I, female predisposition was noticed, where 36 out of the total 78 positive samples (46.15%) were males whereas the remaining 42(53.84%) were females.

Major number of patients out of the 78 Gene Xpert positive samples were in the age group ranging from 21 to 40 years i.e. 36 (46.15%) , followed by 29 (37.17%) in the 41 to 60 years of age followed by minimum number of positive patients were less than 20 years of age.

Table I: Demographic data of the patients with residential area and family size. (n= 863)

Types of samples	Total Number (n=863)	Results of Gene Xpert MTB/RIF			
		DETECTED (78)		NOT DETECTED (785)	
GENDER		**N**	**%**	**N**	**%**
Male	462	36	7.7%	426	92.2
Female	401	42	10.47	359	89.52
AGE IN YEARS					
Up to 20 Years	21	06	28.57	15	71.42
21 to 40 years	367	36	9.8	331	90.19
40 to 60 years	178	29	16.29	149	83.70
>60 years	297	07	2.3	290	97.64
FAMILY SIZE					
< 6	140	23	16.42	117	83.57
>10	723	55	7.6	668	92.39
AREA OF RESIDENCE					
Urban	156	13	8.33	143	91.66
Rural	707	65	9.19	642	90.8%

Maximum number of patients that were reported positive on Gene Xpert were living in the rural part of Swabi, i.e. 65 (83.33%) with maximum number of patients i.e. almost 70.51% living in large families.

Overall Rifampicin resistance as seen on Gene Xpert analysis was 1.28%, as only one of the pus sample out of a total of 78 was detected to be Rifampicin resistant.

Diagnostic performance of Gene Xpert MTB/RIF in comparison with Microscopy was calculated using the 2X2 table, taking Gene Xpert as a standard which showed a sensitivity of 93.58% and specificity of 100%, a positive predictive value was calculated to be 100%, a negative predictive value of 99.36% with an accuracy of 99.42%.

Regarding the sample wise distribution of the patients, as shown in Table II, maximum positive cases i.e 91.02 % of the total 78 positive samples were from the sputum samples and minimum number of cases were found positive from pus i.e. 1.28%. No positive results were detected from both CSF and urine both after Microscopy and Gene Xpert analysis.

Table II also shows a detailed comparison of detection of MTB on Microscopy versus Gene Xpert MTB/RIF. The diagnostic performance of Gene Xpert MTB/RIF was definitely better than Microscopy as was able to detect the presence of MTB in 5 smear negative samples including 3 from pus, and 1 each from sputum and stool.

Diagnostic performance of Gene Xpert MTB/RIF in comparison with Microscopy was calculated using the following 2X2 table, taking Gene Xpert as a standard which showed a sensitivity of 93.58% and specificity of 100%, a positive predictive value was calculated to be 100%, a negative predictive value of 99.36% with an accuracy of 99.42%.

Table III: Diagnostic performance of Microscopy in comparison to Gene Xpert.

	GENE XPERT	
	POSITIVE	NEGATIVE
SMEAR POSITIVE	73 (a)	00 (b)
SMEAR NEGATIVE	05 (c)	785 (d)

Discussion

As reported in our study the overall positivity for Mycobacterium tuberculosis was 9.03%, which was luckily lower than the overall positivity reported from Ethiopia (12.6%)[14] and also lower than a study conducted in Adigrat General Hospital (24.3%)[15] and (26.8%).[16] Also it was reported to be lower from a study conducted in Morocco where they reported the positivity to be (20.59%)[4], Similar to all the above mentioned studies, high positivity was also reported from Nepal i.e. (23.6%)[17] and also in a study conducted in Lahore Punjab where it was (38.0%).[18]

Table II: Sample wise distribution of TB positive samples on Microscopy versus MTB/RIF assay.

Type of sample	Total number of samples tested (n= 863)	Smear positive samples (n=73)	Smear negative samples detected later on Gene Xpert (n=05)	Samples detected positive on Gene Xpert (n=78)
Sputum	751	70 (9.3%)	01 (1.42%)	71 (9.45%)
Fluids	80	01 (1.25%)	0%	01 (1.25%)
CSF	13	00 (0%)	0%	00 (0%)
Pus	11	02(18.18%)	03(27.27%)	05 (45.45%)
Stool	07	00 (0%)	01(14.28%)	01(14.28%)
Urine	01	00 (0%)	0%	00 (0%)

All these studies had reported an overall positivity of MTB detection much higher than the results in our study.

Our study reported maximum number of positive samples i.e. 91.02% being pulmonary in origin, whereas the remaining 8.97% were extra pulmonary in origin.

As reported in our study maximum number of patient who yielded positive results on Gene Xpert/MTB RIF were females, which was in agreement with another study who also reported the same[19], but discordant with another study.[20] The age group mostly affected in our study were between 20 to 60 years of age, which was in agreement with results from another study, [4] although results were variable in different studies[20], showing that any age group /gender could be affected.

Overall Rifampicin resistance as seen on Gene Xpert analysis was 1.28%, as reported in our study, which was lower than a study conducted in the same region of KPK, but another city to be 6.1%[21] and still another study conducted in Lahore, Pakistan with an even higher value of 15.6%. [18]

The diagnostic performance of Microscopy in comparison to Gene Xpert in our study showed a sensitivity of 93.58% and specificity of 100%, which was in accordance with many other studies like a meta-analysis conducted by Shiying Li et al.[22] Other studies from different regions showing different levels of Tuberculosis related endemicity, a sensitivity ranging from 97 to 99%, which is very close to the results in our study and still another study conducted by Opota et al.[23] (2019) found a sensitivity of 100% which is also close to the results in our study.

Similarly, other studies also produced consistent results regarding the specificity of Gene Xpert where it ranges from 73-100%.[2] Osei et al[2], similar to ours in some. Also another study in the year 2019 demonstrated a sensitivity of 50%, and still another conducted in the year 2021 exhibited a sensitivity of 81.6%,[6] which is lower than the sensitivity reported in our study.

As discussed, noticeable degree of variation in the sensitivity and specificity has been reported in different studies, which may be attributed to the presence of PCR inhibitory substances like blood especially in the samples with a paucibacillary disease and destruction of tissues during homogenization. [25,26]

In addition to this, the endemicity rates in different parts of the world, have a substantial effect on the sensitivity and the specificity of Gene Xpert MTB/ RIF which may vary between 82, 98 and 95% for sensitivity, and between 96 and 99% for specificity in areas with low endemicity, whereas in countries with high endemicity, these rates vary between 80 and 88% for sensitivity, and between 95 and 98% for specificity. [22,23]

Based upon the unremarkable performance and short time in which results were provided, Gene Xpert MTB/RIF was recommended by WHO in the year 2010 for diagnosing pulmonary tuberculosis and in 2013 for diagnosing extra pulmonary tuberculosis cases.[27]

Since our study was based on the comparison of the smear microscopy with Xpert MTB/RIF assay in detection of MTB, 73 out of 863 samples were detected positive on smear microscopy. On the contrary Gene Xpert assay detected 78 out of 863 including all true positive cases on smear microscopy and in addition the 05 missed on microscopy, i.e. Gene Xpert MTB/RIF out performed AFB microscopy. Similar results were reported by a study in 2019.[26]

Based on the results in our study, the false negative specimens were higher in EPTB than PTB for the Xpert assay which is evident from the results in another study, since it was evidenced that, the Gene Xpert is associated with variable performance with different samples especially extra pulmonary samples like CSF and urine samples etc as they had lower bacillary burden.[28]

Smear microscopy definitely has the advantage of being rapid, simple and cost effective test, used widely, but as proved in our study it could also be leading to misdiagnosis. Gene Xpert MTB/RIF has proven to be highly specific and sensitive, especially in the smear negative samples.

Conclusion

Gene Xpert MTB/RIF assay has proven to be a highly sensitive user-friendly technique for diagnosing TB and resistance against rifampicin simultaneously for both pulmonary and extra pulmonary TB.

References

1. Tuberculosis Pakistan. WHO Eastern Mediterranean. http://www.emro.who.int/pdf/pak/programmes/stop-tuberculosis.pdf?ua=1
2. Boinwad AS, Iravane JA. Comparison of ZN Microscopy, Culture on LJ Media and Gene Xpert MTB/RIF Assay in Diagnosis of Extra-Pulmonary Tuberculosis. Acta Sci Microbiol. 2021:68-74. doi: 10.31080/ASMI.2021.04.0937.

3. Harirzadeh S, Kazemi MJ, Babakhani S. Identification of Mycobacterium tuberculosis isolated from culture-negative pulmonary and extra-pulmonary samples in cases of suspected tuberculosis. GMS Hygiene and Infection Control. 2019;14.
4. Mechal Y, Benaissa E, El Mrimar N, Benlahlou Y, Bssaibis F, Zegmout A, et al.. Evaluation of GeneXpert MTB/RIF system performances in the diagnosis of extrapulmonary tuberculosis. BMC Infect Dis. 2019; 19:1-8. doi: 10.1186/s12879-019-4687-7.
5. Boonsarngsuk, V., Mangkang, K., and Santanirand, P. (2018). Prevalence and risk factors of drug-resistant extrapulmonary tuberculosis. *Clin. Respir. J.* 12, 2101–2109. doi: 10.1111/crj.12779.
6. Elbrolosy AM, El Helbawy RH, Mansour OM, Latif RA. Diagnostic utility of GeneXpert MTB/RIF assay versus conventional methods for diagnosis of pulmonary and extra-pulmonary tuberculosis. BMC Microbiol. 2021 May 13;21(1):144. doi: 10.1186/s12866-021-02210-5.
7. Matabane MMZ, Ismail F, Strydom KA, Onwuegbuna O, Omar SV, Ismail N. Performance evaluation of three commercial molecular assays for the detection of mycobacterium tuberculosis from clinical specimens in a high TB-HIV-burden setting. *BMC Infect Dis.* 2015;15:508. doi: 10.1186/s12879-015-1229-9.
8. WHO. Global tuberculosis report 2021. https://iris.who. int/bitstream/handle/10665/346387/9789240037021-eng. Pdf-sequence=1 (Accessed: September 14, 2023).
9. Fouda ME, Eman R, Gawad A, Sahar M, Fayed, Mohammad H, et al. A study of the added value of Xpert MTB/RIF assay for assessment of pulmonary tuberculosis transmission risk. Egypt J Med Microbiol. 2019;28(3):141–8.
10. Piatek AS, Van Cleeff M, Alexander H, Coggin WL, Rehr M, Van Kampen S, et al. GeneXpert for TB diagnosis: planned and purposeful implementation. Glob Health Sci Pract. 2013;1(1):18-23.
11. Cepheid. Package insert: Xpert MTB/RIF. https://www.cepheid. com/content/dam/www-cepheid-com/documents/package-insert-files/Xpert-MTB-RIF-ENGLISH-Package-Insert-301-1404-Rev-G.pdf. Sunnyvale, (Accessed: September 14, 2023).
12. The End TB Strategy [website]. Geneva: World Health Organization; 2021 (https://www.who.int/teams/global-tuberculosis-programme/the-end-tb-strategy).
13. Mokobi F. Ziehl-Neelsen Staining- Principle and Procedure with Results [Internet]. Microbe Notes. Sagar Aryal; 2022 [cited 2024 Feb 10]. Available from: https://microbenotes.com/ziehl-neelsen-staining/
14. Araya S, Negesso AE, Tamir Z. Rifampicin-Resistant Mycobacterium tuberculosis Among Patients with Presumptive Tuberculosis in Addis Ababa, Ethiopia. Infect Drug Resist. 2020; 13: 3451–3459. doi: 10.2147/IDR.S263023.
15. Abay G.K., Abraha B.H. Trends of Mycobacterium tuberculosis and rifampicin resistance in Adigrat General Hospital, Eastern zone of Tigrai, North Ethiopia. Trop Dis Travel Med Vaccines 6, 14 (2020). doi: 10.1186/s40794-020-00115-1.
16. Diriba K., Churiso G. The prevalence of Mycobacterium tuberculosis using Gene Xpert among tuberculosis suspected patients in Gedeo Zone, Southern Ethiopia. Eur J Med Res 27, 24 (2022). 10.1186/s40001-022-00650-x.
17. Khadka P., Thapaliya J., Basnet R.B. et al. Diagnosis of tuberculosis from smear-negative presumptive TB cases using Xpert MTB/Rif assay: a cross-sectional study from Nepal. BMC Infect Dis 19, 1090 (2019). doi: 10.1186/s12879-019-4728-2
18. Saeed M, Iram S, Hussain S, Ahmed A, Akbar M, Aslam M. GeneXpert: A new tool for the rapid detection of rifampicin resistance in mycobacterium tuberculosis. J Pak Med Assoc. 2017. Feb; 67(2):270–274.
19. Zahid Q- ul-A, Khursheed N, Adnana F, Zafar A. Concordance and discordance of GeneXpert MTB/RIF and conventional culture method for diagnosis of Extra-Pulmonary Tuberculosis at a tertiary care hospital in Pakistan. Pak J Med Sci [Internet]. 2023Dec.5 [cited 2024Apr.7];40(2(ICON). Available from: https://www.pjms.org.pk/index.php/pjms/article/view/8967.
20. Kabir S, Parash MTH, Emran NA, Hossain ABMT, Shimmi SC. Diagnostic challenges and Gene-Xpert utility in detecting Mycobacterium tuberculosis among suspected cases of Pulmonary tuberculosis. PLoS One. 2021 May 20;16(5):e0251858. doi: 10.1371/journal.pone.0251858.
21. Rahman H, Khan SU, Khan MA, Qasim M, Jabbar A, Noor S, Khan Z, et al. Molecular detection of rifampicin resistance by GeneXpert® assay among treated and untreated pulmonary tuberculosis patients from Khyber Pakhtunkhwa, Pakistan. J Glob Antimicrob Resist. 2017 Jun;9:118-120. doi: 10.1016/j.jgar.2017.02.013.
22. Li S, Liu B, Peng M, Chen M, Yin W, Tang H, et al. Diagnostic accuracy of Xpert MTB/RIF for tuberculosis detection in different regions with different endemic burden: a systematic review and

meta-analysis. PLoS One. 2017;12. https://doi.org/10.1371/journal.pone.0180725.

23. Opota O, Zakham F, Mazza-Stalder J, Nicod L, Greub G, Jaton K. Added Value of Xpert MTB/RIF Ultra for Diagnosis of Pulmonary Tuberculosis in a Low-Prevalence Setting. J. Clin. Microbiol. 2019;57:e01717-18. https://doi.org/10.1128/JCM.01717-18
24. Osei Sekyere J, Maphalala N, Malinga LA, Mbelle NM, Maningi NE. A Comparative Evaluation of the New Genexpert MTB/RIF Ultra and other Rapid Diagnostic Assays for Detecting Tuberculosis in Pulmonary and Extra Pulmonary Specimens. Sci Rep. 2019;9(1):16587. doi: 10.1038/s41598-019-53086-5.
25. National TB Control Programme - Pakistan. Available from: https://ntp.gov.pk/ (Accessed: September 14, 2023).
26. Allahyartorkaman M, Mirsaeidi M, Hamzehloo G, Amini S, Zakiloo M, Nasiri MJ, et al. Low diagnostic accuracy of Xpert MTB/RIF assay for extrapulmonary tuberculosis: A multicenter surveillance. Sci Rep. 2019;9(1):18515. doi: 10.1038/s41598-019-55112-y
27. World Health Organization. Global tuberculosis Report 2018.S.l. WorldHealth Organization; 2018.
28. El-Helbawy RH, Abdel Tawab AM. GeneXpert Mycobacterial tuberculosis/rifampicin: predictors of successful performance. Egypt J Chest Dis Tuberc. 2020;69(1):33-38. doi: 10.1186/s12866-021-02210-5