RESEARCH ARTICLE

Comparison evaluation between gene Xpert Mtb/Rif and multiplex PCR for rapid diagnosis of mycobacterium tuberculosis

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Abstract

Objective: To compare the efficacy of Gene Xpert mycobacterium tuberculosis-rifampicin and multiplex polymerase chain reacton for the detection of mycobacterium tuberculosis and Rifampicin resistance.

Method: The cross-sectional validation study was conducted at the Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi, Pakistan, from March to October 2018, and comprised mycobacterium tuberculosis-positive rifampicin-resistant and rifampicin-susceptible samples, with the latter acting as negative controls. Gene Xpert mycobacterium tuberculosis-rifampicin assasy and multiplex polymerase chain reacton were applied simultaneously and compared with gold standard mycobacterium growth indicator tube 960. Data was analysed using SPSS 24.

Results: Of the 192 samples, 84(44%) were culture-positive rifampicin-resistant and 108(56%) were culture-positive rifampicin-susceptible. Overall, 84(44%) were found positive. Gene Xpert mycobacterium tuberculosis-rifampicin assay detected all 84(100%) rifampicin-resistant samples, while multiplex polymerase chain reacton detected 44(52.3%) such samples. Sensitivity, specificity, positive predictive value and negative predictive value of Gene Xpert were 100% each respectively, while the corresponding values for multiplex polymerase chain reacton were 52%, 100%, 100% and 72% respectively.

Conclusion: Molecular detection of mycobacterium tuberculosis and resistance by Gene Xpert and multiplex polymerase chain reacton simultaneously was found to be a rapid and cost-effective method.

Keywords: RIF, Gene Xpert MTB / RIF, Multiplex PCR. (JPMA 71: 636; 2021)

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Introduction

The incidence of drug-resistant tuberculosis (DR-TB) is rising in Asia and Africa.[1,2] According to the World Health Organization (WHO), 10.4 million individuals suffered from TB and 1.8 million deaths were reported due to it.[3] The WHO 2018 Global TB Report revealed that 3.5% of new cases and 18% of existing ones were due to multi-drug resistant (MDR) or Rifampin (RIF)-mono-resistant strains.[4] MDR-TB results from inappropriate treatment, poor compliance, lack of patient education, strain characteristics and nosocomial characteristics.[5] Diagnosis of TB is based on clinical, radiological and laboratory investigations, including microscopy using acid-fast bacillus (AFB) staining etc, gold standard culture and molecular diagnosis using nucleic acid amplification (NAA).

For a quick diagnosis of MDR-TB and mono-resistant-TB, latest modalities like NAA are used. Two methods are used; probe-based non-sequencing, and sequencing type test.[6,7] Non-probe-based sequencing tests are accurate and detect drug resistance with greater and effective manner than probe-based non-sequencing test. Another method approved by the Food and Drug Administration (FDA) and endorsed by the WHO in 2011 was Gene Xpert mycobacterium tuberculosis (MTB) / RIF and later in 2017 Gene Xpert Ultra was introduced for better outcomes.[8] Gene Xpert MTB/RIF is simple and easy to perform with no cross contamination and good impact on resource poor regions.[9] Mutation due to rpo B gene mainly attributed rifampin resistance proved by genotypic test like polymerase chain reaction.[10,11] In this study multiplex PCR was performed to detect MTB and mutated rpo B gene of RIF along with Gene Xpert MTB / RIF.

The current study was planned to compare multiplex polymerase chain reacton (PCR) and Gene Xpert in the diagnosis of DR-MTB while keeping culture as the gold standard.[12]

Materials and Methods

The cross-sectional validation study was conducted at the Department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi, Pakistan, from March to October 2018. After approval from the institutional ethics review committee, the sample size was calculated with WHO calculator using the formula: N=z2p(1-p)/e; where z =

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confidence interval (CI), p = prevalence and e = error in CI.[13] The sample was raised using non-probability sampling technique from the AFIP repository. All pulmonary samples, like sputum, broncho-alveolar lavage (BAL) and pleural fluid, as well as extra-pulmonary samples, like pus, tissue, cerebrospinal fluid (CSF) and bone marrow, of the diagnosed patient of TB were included, and so were anti-TB drug sensitive samples which were used as negative control. Repeated samples and mycobacterium other than tuberculosis (MOTS) were excluded.

All the samples were digested, decontaminated and concentrated by sodium hydroxide (NAOH) and N-acetyl-L-cysteine (NALC) to reach the final concentration of 2% as NAOH, followed by vortex and centrifugation 3000 x g for 15-20 min and subsequently subjected to AFB staining. All the specimens were processed for AFB staining quantitatively and the number of live bacteria present in smear. Mycobacterium growth indicator tube (MGIT) is composed of modified Middlebrook 7H9 broth, growth supplement composed of oleic acid, albumin, dextrose and citrate (OADC) with another solution comprising polymixin B, amphotericin B, naldixic acid, trimethoprim and azlocillin (PANTA) in conjugation with a fluorescence quenching-based oxygen sensor. When bacteria had consumed oxygen, the amount of oxygen depleted and the indicator at the bottom of the tube illuminated with ultraviolet (UV) light. All the culture-positive samples were further processed for drug susceptibility to detect the antibiotic resistant for RIF, isoniazid (INH), ethambutol (EMB) and pyrazinamide (PZD) using BACTEC MGIT SIRE kit (Becton, Dickinson)[14,15] according to the manufacturer's instruction. Positive control for MGIT 960 system is ATCC25177 MTB, and un-inoculated MGIT tube is a negative control for the detection of organisms. AFB staining was done when the signal was positive by MGIT 960 after scanning a barcode. To differentiate between MTB and MOTS, rapid immuno-chromatographic BD MGIT TBc identification test was performed. It is an automated cartridge-based method to detect MTB complex along with RIF resistance simultaneously in primary TB samples, including smear-positive, smear-negative and cultured samples, using real time PCR assay. This method detects MTB along with RIF resistance rpoB gene mutation at 81-bp size, which is the most common RIF resistance determining region of mutation. Further, 1ml of samples was taken and transferred to screw-capped tube containing 3ml reagent at a ratio 1:3 according to the manufacturer's instruction. The reagent was inactivated with isopropanol and NAOH then vortexed and left at room temperature for 15 minutes. After mixing well until liquified, it was transferred into Xpert MTB / RIF cartridge which also included internal control for sample processing. The cartridge was placed into Gene Xpert instrument and results were available in <2 hours. For multiplex PCR deoxyribonucleic acid (DNA) was extracted by 250µl of cell lysis solution placed in Eppendorf tube already containing 50µl TB samples and incubated at 65°C for 15 minutes. Further, 100µl protein-precipitating solution was added to the same tube followed by vortex and centrifugation at 1500xg /15 min. Supernant was discarded from the tube and DNA pellet was obtained. Ethanol 100% and isopropanol were added according to the manufacturer's instruction. Finally, 10µl DNA hydration solution was added and incubated for 7 minutes at 65°C.

Pairs of primers were used: Forward: CAGACGTTGATCAACATCCG, Reverse: TACGGCGTTTCGATGAAC and Forward: CGGCGATGAGCGTTACA, Reverse: CGTCCTTGGCGGTGTATT[16] (Table-1). They were selected against RIF and INH drugs against rpoB and KatG genes simultaneously (Table-2). The melting temperature was 58°C.

Table-1: Primer selection.

Anti-TBdrugs	Target sites	Primers	Tm	bp	Reference
Rifampin (RIF)	rpo B gene(531)	F:CAGACGTTGATCAACATCCG R:TACGGCGTTTCGATGAAC	58°C	305	(13)
Isoniazid (INH)	Kat G(315)	F: CGGCGATGAGCGTTACAC R: CGTCCTTGGCGGTGTATT	58°C	458	(13)

Table-2: Polymerase chain reacton (PCR) mixture.

Reagents	Concentration	Volume
DNA	1-4ng	2µl
Taq Polymerase	0.5 µmol	0.5µmol
dNTPS	0.3µmol	0.3µmol
MgCl2	0.2µmol	0.2µmol
Buffer without MgCl2	3µl	3µl
Distilled water		15µl
Forward primers for rpoB and katG genes	1µmol	2µl
Reverse primers for rpoB and katG genes	1µmol	2µl
Total		25-28µl

DNAL Deoxyribonucleic acid; dNTPS: Deoxyribonucleotide triphosphate; MgCl2: Magnesium chloride.

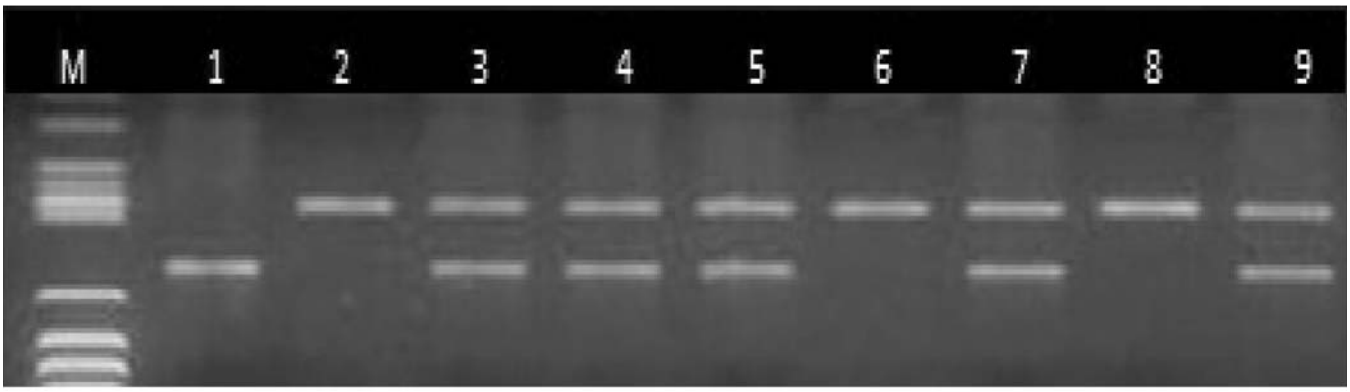

Figure: Characteristic banding pattern obtained for Rifampin-resistant strain using Multiplex polymerase chain reacton (PCR).

The PCR reaction mixture placed in the Eppendorf tube was run in thermocycler machine with initial and final denaturation of 5 min at 95°C, annealing at 58°C for 30 seconds, polymerisation at 72°C for 45 seconds followed by extension at 72°C for 7 min.

For gel electrophoresis, 1% agarose gel was used in multiplex PCR. The run-off time was 1-1.5 hours with 120 voltage. DNA bands were visualised under UV light. A single band of 305bp specified resistance to RIF. A single band of 458bp specified resistance to INH while using ladder 100bp as control (Figure).

Data was analysed using SPSS 24. Statistics including sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV) of Gene Xpert / RIF and multiplex PCR were calculated.

Results

Of the 192 samples, 84(43.75%) were pulmonary (60[71.4%] sputum, 20[23.9%] endometrial biopsy [EB] washings, 4[4.7%] BAL) and 108(56.25%) extra-pulmonary (71[65.7%] tissue, 25[23.1%] pus and 12[11.1%] CSF). The mean age of patients was 40±17 years; 100(53%) males and 92(47%) females. Of the 84(43.75%) culture-positive patients, MTB/RIF test identified 79(94%) with smear-positive TB, and of the 108(56.25%) smear-negative cases, it identified 5(4.6%). Overall, there were 108(56.25%) samples susceptible for RIF and were true negatives (100[92.6%] susceptible to RIF and 8[7.4%] culture-negative). Of the remaining 84(43.75%)culture-positive RIF-resistant samples, 40(47.6%) related to sputum, 5(6%) EB washings, 1(1.1%) BAL, 30(35.7%) tissue, 6(7.1%) pus and 2(2.4%) CSF. All the 84(100%) culture-positive RIF-resistant true positive samples were detected by Gene Xpert/RIF, while multiplex PCR detected 44(52.38%) of them and 40(%)were false negative.

Sensitivity, specificity, PPV and NPV of Gene Xpert were 100% each respectively, while the corresponding values for multiplex PCR were 52%, 100%, 100% and 72% respectively (Table-3).

Discussion

TB has a very high prevalence among resource-poor countries like Pakistan. Early diagnosis and its effective treatment play a major role in breaking the chain of transmission and pathogenesis. There are different modalities to diagnose this deadly disease. From simple microscopy to gene sequencing, various tools are available for diagnosis. The culture and drug susceptibility test remains the gold standard as it can detect 10-100 organisms, strain typing, speciation identification, mixed infection detection and quantification of growth.[17]

The current study compared Gene Xpert/RIF and multiplex PCR for the rapid diagnosis of MTB and RIF resistance using the gold standard MGIT culture method. Advantages of

Table-3: Sensitivity, Specificity, Positive predictive value (PPV), negative predictive value (NPV) of Gene Xpert / Rifampicin (RIF) and Multiplex polymerase chain reacton (PCR) (n=192).

	Culture Resistant	susceptible	Sensitivity(%)	specificity(%)	PPV (%)	NPV (%)
Gene Xpert/RIF						
Resistant	84	0	100%	100%	100%	100%
Susceptible	0	108				
Multiplex PCR						
Resistant	44	0	52%	100%	100%	72%
Susceptible	40	108				

molecular methods are early diagnosis, determination of resistance pattern, start of empirical therapy to patients, physician guidance for MDR and extensively drug resistant (XDR) TB among patients in the early stage.

Gene Xpert MTB/RIF showed sensitivity and specificity of 100%. These results were in concordance with earlier studies.[18,19]

Multiplex PCR is conventional PCR which can be used as a cost-effective and quick method for the detection of RIF resistance and MDR-TB in resource-limited setups. Multiplex PCR in the current study showed sensitivity 51% and specificity 100%.[20] A study on multiplex PCR used RIF mutated gene rpoB at codon 531, which is the most common mutation In 85% cases. Sensitivity of RIF resistance varies from region to region and it is said to be the most common mutation of first-line anti-TB drugs. In the current study, out of 84 culture-positive samples, 17 were RIF-resistant and remained positive to both Gene Xpert MTB / RIF and multiplex PCR. The finding is in line with an international study and one that was done regionally.[21,22]

Conclusion

Gene Xpert MTB/RIF and multiplex PCR were found to be time-saving, less laborious, cost-effective tools than culture and drug susceptibility testing for TB patients. Gene Xpert MTB/RIF was found to be a more sensitive and specific method than multiplex PCR.

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Conflict of Interest: None.

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