Case Report

Pyrosequencing to resolve discrepant Xpert MTB/RIF and Mycobacterial Growth Indicator Tube 960

Kanchan Ajbani, Mubin Kazi, Jeffrey Tornheim[1], Swapna Naik, Rajeev Soman[2], Anjali Shetty, Camilla Rodrigues

Departments of Microbiology and [2]Medicine, P. D. Hinduja Hospital and Medical Research Centre, Mumbai, Maharashtra, India, [1]Johns Hopkins University School of Medicine, Division of Infectious Diseases, Center for Clinical Global Health Education, Baltimore, MD USA

ABSTRACT

Delayed diagnosis of drug resistance has been a major obstacle to proper management and control of drug-resistant tuberculosis (TB). Expanded access to rapid molecular diagnostics such as Xpert MTB/RIF has been helpful, but has generated confusion about how to interpret genotype–phenotype discordance. Optimal management is not clearly defined for patients with rifampin resistance by Xpert MTB/RIF but rifampin susceptibility by phenotypic testing. To resolve this discrepancy, we performed pyrosequencing of discordant isolates identified at a reference laboratory over a 6-month period. We present here strategies to address genotype–phenotype discordance using sequencing.

KEY WORDS: Culture, discrepancy, pyrosequencing, Xpert

Address for correspondence: Dr. Camilla Rodrigues, Department of Microbiology, P. D. Hinduja Hospital and Medical Research Centre, Veer Savarkar Marg, Mahim (West), Mumbai - 400 016, Maharashtra, India. E-mail: dr_crodrigues@hindujahospital.com

Delayed diagnosis of drug-resistant tuberculosis (TB) has been a major obstacle to the correct management and control of the disease. To address this problem, the WHO has endorsed the use of rapid molecular tests (Xpert MTB/RIF and the MTBDRplus line probe assay) for the diagnosis of TB and the detection of drug resistance to rifampicin (RIF) in developing and high-burden countries.[1] Despite the WHO endorsement of molecular tests, phenotypic drug susceptibility testing (DST) is still considered the gold standard. As knowledge of molecular tests has increased, it has been observed that the gold standard is not "solid gold" and TB isolates are identified as susceptible by phenotypic DST despite harboring specific disputed mutations in the *rpoB* gene (the gene responsible for resistance to RIF).[2-5] As laboratory capacity for TB diagnostics expands in developing countries, discrepant genotypic and phenotypic results will become increasingly apparent.

While highly sensitive, Xpert MTB/RIF cannot differentiate between genetic changes that impact protein structure and those that do not ("silent" mutations), which can result in discordance between testing methods that is difficult to interpret. Pyrosequencing (PSQ) offers the opportunity to differentiate these discordant samples by identifying which specific mutation is present. PSQ is a real-time method that rapidly sequences small segments of genomic DNA and reliably detects mutations that confer first- and second-line drug resistance in *Mycobacterium tuberculosis*. PSQ not only determines the presence or absence of these mutations but also displays the detailed sequence data, which enables users to distinguish mutations conferring resistance from silent mutations as well as from those conferring different degrees of drug resistance.[6]

At our private tertiary care hospital in Mumbai with a referral bias toward nonresponders, we reviewed laboratory records for genotypic and phenotypic results from all discrepant isolates found to be resistant by Xpert

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MTB/RIF but sensitive by phenotypic DST between June 2015 and December 2015 [Table 1]. We have studied 94 isolates where both DST and Xpert were available, of which these 9 were discrepant.

Of the 9 Xpert MTB/RIF resistant isolates we analyzed, 4 failed to hybridize Xpert MTB/RIF probe A, 4 failed to hybridize Xpert MTB/RIF probe E, and 1 failed to hybridize Xpert MTB/RIF probe D. All isolates were susceptible by mycobacterial growth indicator tube (MGIT) DST at the critical concentration of 1 μgm/ml. All of these discordant isolates were submitted for PSQ.

Sequencing of these 9 samples identified mutation CTG511CCG (Leu511Pro) for all of the isolates missing probe A, CAC526AGC (His526Ser) for the isolate missing probe D, and CTG533CCG (Leu533Pro) for all missing probe E. Data were compared to published minimum inhibitory concentration (MIC) values for these sites to determine likely susceptibility [Table 1].

The use of PSQ in our laboratory helped clarify discordant results among 9 isolates of TB. Each of the three mutations identified in the *rpoB* gene (CTG511CCG, CAC526AGC, and CTG533CCG) are considered disputed mutations which are known to be associated with variable susceptibility results in growth-based assays[7-9] but have been reported to be clinically significant. In a 2013 study evaluating samples from two countries with a high burden of drug-resistant TB, disputed rpoB mutations were responsible for over 10% of RIF resistance among first-line failure and relapse cases.[5,10] Our data are consistent with other studies wherein disputed mutations could result in low-level resistance to RIF that is not identified by RIF MGIT 960 DST.[11] To address the discrepant Xpert MTB/RIF and MGIT DST, we propose the algorithm as shown in Figure 1 using PSQ to resolve the discrepancy. This approach addresses synonymous (silent) mutations that are flagged as resistant by Xpert MTB/RIF but do not translate to amino acid modification. One arm of the algorithm suggest to look for dual population (i.e., heteroresistance[12]), but among in this group of discrepant isolates, we did not see any probable heteroresistance population. The algorithm also proposes to use RIF MIC that helps clarify discrepancies. The basic idea of the algorithm is to resolve the discrepancy that might arise between phenotypic testing (MGIT-DST) and GeneXpert, wherein any sequencing technique is recommended. This approach is consistent with CDC recommendations for the resolution of genotype–phenotype discordance and may help identify which discordant isolates are truly resistant to RIF based on MIC range predicted for a particular mutation.[13] We estimate that this approach will only become more important in the coming years as PSQ and whole-genome sequencing become more broadly diagnostic tools at referral laboratories in India. Any sequencing-based technique would help identify the exact single-nucleotide polymorphisms to resolve any discrepancy.

Table 1: Mutations identified among Xpert MTB/RIF resistance detected - mycobacterial growth indicator tube drug susceptibility testing rifampicin susceptible tuberculosis isolates collected over 6 months at Hinduja Hospital, Mumbai

Number of discordant isolates	Xpert probe missing	PSQ mutation identified	Literature-derived MIC (μg/ml)	Reference
4	A	CTG511CCG	≤0.125-0.5	[11,14,15]
1	D	CAC526AGC	0.5-1	[16]
4	E	CTG533CCG	2.0	[15]

PSQ: Pyrosequencing, MIC: Minimum inhibitory concentration

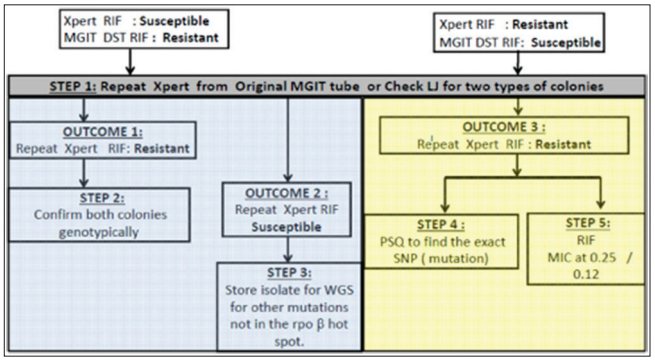

Figure 1: Algorithm for discrepant Xpert MTB/ RIF and mycobacterial growth indicator tube drug susceptibility testing rifampicin

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Conflicts of interest

There are no conflicts of interest.

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