REVIEW ARTICLE

Line Probe Assay for Rapid Diagnosis of Multidrug Resistant Pulmonary Tuberculosis

Nancy A. Al-Bassuony, Wafaa S. Zagloul, Mohammad A. Abou Elela, Mohamed K. Elbadrawy, Dalia M. Moemen

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ABSTRACT

Multidrug-resistant tuberculosis (MDR-TB) is a serious public health problem with ultimate concern in the developing countries, including Egypt. In the global TB report issued in 2021 by World Health Organization (WHO), infection caused by the tubercle bacilli Mycobacterium tuberculosis (MTB) represents the 13th chief cause of mortality worldwide. Effective MDR-TB control counts on the accuracy and rapidity of its diagnosis in order to start the proper treatment regimen and as a result limit the transmission of the resistant isolate. Line probe assay (LPA) and GeneXpert MTB/RIF are two WHO approved probe based molecular assays for rapid detection resistant MTB isolates via screening its nucleic acid for any mutations. For the purpose of finding mutations linked to rifampicin resistance using DNA probes, both techniques focus on the same 81 bp Rifampicin Resistance Determining Region. Line probe assay can detect both rifampicin and isoniazid resistance, thus can detect isoniazid mono resistant isolates, which requires special management protocol, in addition to detecting MDR-TB isolates as well with high sensitivity and specificity.

INTRODUCTION

Tuberculosis (TB) is a serious infection, caused by the tubercle bacilli MTB which is the ninth chief cause of mortality worldwide also the leading cause from a single infectious agent and is anticipated to withstand its current position or even ascent to the seventh position.

In spite the fact that Egypt is not found within the World Health Organization (WHO) list of the high burden TB countries, Egypt is considered within the highest percentage in Eastern Mediterranean countries where MTB is the third most significant communicable infection after hepatitis C and bilharziasis.

Multidrug-resistant tuberculosis (MDR-TB) has emerged as a major health concern around the globe. Alarming reports are arising from several parts all over the world of increasing drug resistance, which potentially threaten disrupting achievements gained in TB control over the last decade.

Although mycobacterial culture is considered as the gold standard by WHO for both MTB diagnosis and drug susceptibility testing (DST) nonetheless it is slow and requires high technical expertise. Thus, standardized and optimized MTB culture and DST is difficult as it requires several weeks to months for laboratory results to become available, during which, patients may be prescribed inadequate treatment, hence increasing the development and spread of resistant isolates.

Therefore, there is an urgent necessity for developing accurate and prompt DST for MDR-TB to avoid clinical deterioration, improve treatment regimen, and interrupt further transmissions. Since MTB resistance to the treatments is triggered by genetic mutations in the drug targets genes, either in regulatory regions of the target gene or in drug-activating genes, the molecular diagnosis is considered the method of choice for prompt identification of MDR-TB.

Multidrug resistant tuberculosis

Multidrug-resistant Tuberculosis is caused by TB strain that are resistant to at least isoniazid and rifampicin, the two most potent TB drugs. MDR-TB has been an area of growing concern to human health worldwide which is hazardous to the TB control.

Over the years poor chemotherapy resulted in selective evolution of resistant mutants. The incidence of resistant cases mainly depends on the ratio between native bacilli and the resistant mutants in the lesion. However, inadequate therapy, interrupted treatment, inappropriate drug doses or addition of a single drug to a failing regimen allows selective growth of resistant mutants and acquisition of DR-TB.

The history of resistance in MTB is a recent issue, which emerged over the past sixty years accompanied by the anti-tuberculosis drugs development. With the
discovery of rifampicin in 1966 and its extended use between 1970 and 1990, patients who were carriers of isoniazid resistant strains became rifampicin resistant. This marked the beginning of an MDR-TB problem that has since developed into a pandemic in several nations.

Also, it was shown that there is a direct correlation between strong National TB Programmes (NTP) practices and low resistance rates. According to recent evidence, NTPs that effectively utilize currently available medications may be able to delay or perhaps decrease the DR-TB prevalence. Table (1) lists the commonest reasons for resistance in the general population and how MDR-TB develops during epidemics.

Transmission of MDR-TB depends on both the virulence of the drug-resistant strain and the susceptibility of the population. The prevalence of DR-TB is correlated with 1) previous TB management, 2) current TB management, and 3) MDR-TB transmission. However, there are many factors besides these three that affect the epidemiology of MDR-TB, therefore the anticipated changes in both TB and MDR-TB prevalence varies greatly.

Table 1: Most frequent causes associated with selection of resistance in the community and generation of DR-TB.

<table>
<thead>
<tr>
<th>Non-implementation of DOTS and DOTS expansion strategies</th>
<th>Inadequate supply or poor quality of drugs</th>
<th>Patients: inadequate drug intake</th>
<th>Others</th>
</tr>
</thead>
<tbody>
<tr>
<td>Poorly organized or funded NTPs</td>
<td>History of frequent drug supplies shortages in the country</td>
<td>Inadequate adherence to treatment</td>
<td>Dominant private sector</td>
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<tr>
<td>Inadequate or missing guidelines</td>
<td>Poor quality of anti-TB drugs</td>
<td>Adverse effects and malabsorption</td>
<td>Poor infection control practice</td>
</tr>
<tr>
<td>Poor training</td>
<td>Wrong dose or combination</td>
<td>Social barriers</td>
<td>High prevalence of highly virulent DR-TB strain</td>
</tr>
<tr>
<td>Lack of treatment monitoring</td>
<td></td>
<td>Financial problems (treatment not for free)</td>
<td>HIV infections</td>
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<tr>
<td>Non-standardized treatment</td>
<td></td>
<td>Substance dependency disorders</td>
<td></td>
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</table>

Anti-TB drug resistance screening was considered important and necessary on worldwide. Seventy-one percent of those with bacteriologically proven PTB in 2020 had their rifampicin resistance evaluated, according to an official WHO report in 2021: 61% in 2019 and 50% in 2018. There are ten countries that account for about 70% of the MDR-TB global burden in 2020: China, Democratic Republic of the Congo, India, Indonesia, Nigeria, Pakistan, Philippines, Russian Federation, South Africa and Viet Nam.

Molecular diagnosis of MDR-TB

For several ages, patients with un-diagnosed DR-TB who have received the standardized treatment regimen, accelerated the emergence of MDR-TB through drug-specific resistance augmentation. Comprehensive drug susceptibility testing (phenotypic and/or genotypic) is a need to inform physicians about the best treatment regimen tailored individually according to patient susceptibility results.

The phenotypic drug susceptibility testing (DST) is considered the gold standard for the diagnosis of DR-TB. Nonetheless, this method is time-consuming (takes up to 3–8 weeks).

Previous studies verified most spontaneous mutations, particularly single nucleotide polymorphisms (SNPs) on the circular chromosome, are the chief cause of treatment resistance in MTB strains. Each antitubercular drug is associated with one or more genetic mutation which is responsible for variable degrees of resistance with variable frequencies.

For example, rpoB gene mutations, primarily in an eighty-one bp hotspot region, are responsible for 97% of cases of resistance to rifampicin (codon 507 to codon 533), however the highest frequency and highest-level of rifampicin resistance is conferred to mutations at codons 526–531 of rpoB. Also, isoniazid resistance is caused by mutations in the inhA, katG, ahpC, ndh, and furA genes. The majority of katG mutations are found at codon 315, which is responsible for increasing isoniazid resistance by 50% up to 90%.

World Health Organization has authorized certain molecular diagnostic tests listed in Table (2) for detection of TB and drug-resistant strains to overcome the drawbacks of conventional DST techniques, to allow rapid diagnosis of DR-TB without any delay which is a corner stone step in the control of MDR-TB.

Molecular drug susceptibility techniques are more rapid and accurate than the conventional phenotypic tests. Molecular techniques can diagnose both MTB and the associated drug resistance through nucleic acid amplification tests.
Line probe assays (LPAs):

Line probe assays are DNA hybridization assays that can detect different mutations at the same time by using multiple probes. After DNA extraction and target amplification, amplicons are hybridized to specific oligonucleotide probes that are complementary to the target sequences and are immobilized on the surface of a strip. After several post-hybridization washes to remove non-specific binding, the amplicon-probe hybrids are visualized by eye as colored bands on the strip. The turnaround time of the whole assay is 5–7 h.

Line probe assays are rapid molecular tests that can diagnose MTB and drug resistance, even though LPAs are designed for reference or regional laboratory settings, because they are more technically complex and take longer time to perform, however LPAs have an advantage over the Xpert MTB/RIF assay, they are able to detect both isoniazid and rifampicin resistance, in addition to second line injectables and fluoroquinolone.

Principle of Line probe assays:

Line probe assays are a group of strip-based DNA tests which detect drug resistance profile of an MTB isolate through the binding pattern of amplicons to probes targeting the commonest mutations associated with first and second-line agents resistance as well as to probes targeting the wildtype (WT) DNA sequence. Mutation are detected either though binding of biotin-labeled amplicons to the target mutations, or the absence of the corresponding wild type probes hybridization washes.

First Line- Line Probe Assay:

The WHO endorsed LPAs for MTB and rifampicin resistance detection in smear-positive TB cases in 2008, at the beginning Genotype MTBDR assay (Hain Lifescience, Nehren, Germany) and INNO-LiPA Rif.TB assay (Innogenetics, Ghent, Belgium) were initially used, later it was replaced by more recent versions of the LPA technology.

Further researches have been issued and authorized by the WHO toward guiding an updated policy for using molecular-based diagnostic assays, where the performance of three LPAs were assessed: GenoType MTBDR plus version 1 and 2, and Nipro NTM+MDRTB Detection Kit 2, for screening isoniazid and rifampicin resistant MTBisolates.

Nipro NTM+MDRT-B distinguishes four significant Mycobacterium species (M. tuberculosis, M. avium, M. intracellulare, and M. kansasii) that cause the human

Table (2): WHO-authorized Molecular Drug-Susceptibility tests

<table>
<thead>
<tr>
<th>Assay</th>
<th>Drug Resistance Sensitivity, Specificity %</th>
<th>DR-TB Category</th>
<th>Time to Result</th>
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<tbody>
<tr>
<td>GeneXpert assays</td>
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<tr>
<td>XPERT MTB/RIF (Cepheid) 2010</td>
<td>RIF (96, 98)</td>
<td>RR-TB</td>
<td>2 hours</td>
</tr>
<tr>
<td>XPERT MTB/RIF Ultra (Cepheid) 2017</td>
<td>RIF (94, 98)</td>
<td>RR- TB</td>
<td>2 hours</td>
</tr>
<tr>
<td>XPERT MTB/XDR (Cepheid) 2021</td>
<td>INH (94.2, 98); ETH (98, 99.7); FQ (93.1, 98.3); AMK (86.1, 98.9); CAP (70, 99.7); KAN (98.1, 97)</td>
<td>RR- TB; MDR- TB; Pre- XDRTB</td>
<td>1.5 hours</td>
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<tr>
<td>Truenet assay</td>
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<tr>
<td>TRUENAT MTBRI/F/Dx (Molbio) 2020</td>
<td>RIF (84, 97)</td>
<td>RR- TB</td>
<td>1 hour</td>
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<td>Moderate complexity automated NAATs</td>
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<tr>
<td>RealTime MTB RIF (Abbott) 2019</td>
<td>RIF (94.8, 100); INH (88.3, 94.3)</td>
<td>RR-TB; MDRTB</td>
<td>10.5 hours</td>
</tr>
<tr>
<td>BD MAX MDR-TB (Becton Dickson) 2021</td>
<td>RIF (90, 95); INH (82, 100)</td>
<td>RR-TB; MDRTB</td>
<td>4 hours</td>
</tr>
<tr>
<td>Cobas MTB-RIF/ INH (Roche) 2021</td>
<td>RIF (97.2, 98.6); INH (96.9, 99.4)</td>
<td>RR-TB; MDRTB</td>
<td>4.5 hours</td>
</tr>
<tr>
<td>FluoroType MTBDR (Hain) 2021</td>
<td>RIF (98.9, 100); INH (91.7, 100)</td>
<td>RR-TB; MDRTB</td>
<td>3 hours</td>
</tr>
<tr>
<td>Line probe assay</td>
<td></td>
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</tr>
<tr>
<td>GenoType MTBDRplus (Hain) 2008</td>
<td>RIF (98.2, 97.8); INH (95.4, 98.8)</td>
<td>RR-TB; MDRTB</td>
<td>5-7 hours</td>
</tr>
<tr>
<td>Genoscholar NTM/MDRTB strip (Nipro) 2012</td>
<td>RIF (100, 95); INH (97, 95)</td>
<td>RR-TB; MDRTB</td>
<td>5-7 hours</td>
</tr>
<tr>
<td>GenoType MTBDRSl (Hain) 2016</td>
<td>FLQ (100, 98.9); AMK (93.8, 98.5); CAP (86.2, 95.9)</td>
<td>RR-TB; MDRTB</td>
<td>5-7 hours</td>
</tr>
</tbody>
</table>

Abbreviations: RR-TB, rifampicin-resistant tuberculosis; MDR-TB, multidrug-resistant tuberculosis; N/A, not applicable; RIF, rifampicin; INH, isoniazid; FLQ, fluoroquinolone; AMK, amikacin; KAN, kanamycin; ETH, ethionamide; CAP, capreomycin
disease in addition to detecting MDR-TB cases by targeting rpoB, katG, and inhA. Although the sensitivity of the Nipro strips varies between studies (from 50 to 95%), they have a good specificity for isoniazid and rifampicin resistance screening between 97 and 100% in both clinical samples and cultured isolates. On the other hand, GenoType MTBDR plus version 2 sensitivity varies between 83.3 and 96.4%, and specificity between 98.6 and 100%.  

Second Line LPA:

In May 2016, WHO approved this rapid diagnostic test called MTB DRsl (SL-LPA) a DNA-based test that identifies mutations within MDR/Rifampicin resistant-TB strains, responsible for fluoroquinolone and injectable second-line TB drugs resistance. It is the initial and only WHO-recommended assay for identifying new resistance in MDR and XDR-TB without delay. It provides results within 1-2 days, which is a chief advantage over the 3 months or longer results obtained by phenotypic DST.

However, adoption of SL-LPA does not eliminate the importance of conventional culture and DST. Despite good specificity of SL-LPA in detecting resistance to fluoroquinolone and the second-line injectable drugs, culture and phenotypic DST are required to completely exclude resistance to these drug classes along with other second-line drugs.

In conclusion, LPAs are simple and easy assay to perform and straightforward to interpret which can be performed either directly from respiratory samples or indirectly from culture material with short turnaround time to detect isolates with isoniazid and rifampicin resistance, allowing early appropriate treatment, which reduces transmission and spread of MDR-TB.

However, some mutations that confer resistance are outside the regions covered by the test therefore resistance cannot be fully excluded even if all WT probes are present. Also, LPA is less efficient than conventional DST in detecting resistance in samples comprising hetero-resistance strains. More precisely, with LPA, it is possible to detect resistant bacteria with mutations detected by the MUT probes if resistant bacteria represent at least 5% from entire population. Still, resistant bacteria with mutations inferred by the absence of WT probes would be missed if the resistant population is less than 95% from the entire population.

Potential clinical and epidemiologic relevance of LPA use in practice

The main pillar of integrated patient centered care and prevention within the Strategy by End TB committee has stated the need for the early TB detection including anti-tuberculous sensitivity testing. This emphasizes how crucial LPAs are for the quick detection of MTB and its resistance pattern. As well, the International Standards for TB Control recommends LPA for MDR-TB diagnosis, especially in environments where the risk of isoniazid mono-resistance is high.

Currently available commercial LPA detect mutations in rpoB codons 516, 526 and 531. There is a high-level agreement between genotypic and phenotypic DST. This is because the mutations associated with rifampicin resistance are located within 81 bp core region of rpoB, while mutations outside this region are unusual. Molecular based techniques for screening rifampicin resistance could be considered a standard for the diagnostic evaluation of presumptive MDR-TB cases.

Although, majority of rifampicin resistant strains are also resistant to isoniazid, molecular testing for isoniazid drug resistance is important as it offers the possibility of adding isoniazid to a second-line drug regimen, in addition the management of rifampicin resistant TB is different if accompanied by isoniazid resistance.

As for isoniazid resistance LPA detect mutations in inhA positions 16, 15 and 8, and katG codon 315. High-level isoniazid resistance is associated with S315T mutation without affecting ethionamide susceptibility. The limited data on the direct association between katG S315T mutation and clinical outcome suggest increased risk of first-line treatment failure, death and relapse. In the case of katG S315T mutation, isoniazid should therefore be excluded from treatment. Compared to katG S315T, inhA promoter mutations confer low-level isoniazid resistance, but significantly affect ethionamide susceptibility.

Evidence which supports continuing of using LPAs was provided based on data collected from a variety of laboratory settings in various countries, based on their diagnostic accuracy when used directly on sputum smear-positive specimens or indirectly from culture isolates, as an initial test or in conjunction with culture-based DST for the detection of MTB and MDR. In countries with middle or low incidence, LPAs may also be a vital tool in the efforts exerted to eradicate TB.

Based on recent MDR-TB treatment guidelines, isoniazid resistance does not prevent its use, as the usefulness of high-dose isoniazid in patients infected with strains harboring mutations either within inhA promoter or region katG is uncertain, taking in consideration that inhA mutant strains are naturally resistant to ethionamide and prothionamide.

Hence, identifying strains with isoniazid resistance by applying first line LPAs will be valuable to clinicians, if it provides the results quickly and does not cause any delay the start of therapy.
CONCLUSION

Line Probe assay application in MDR-TB early diagnosis of will provide rapid and more accurate identification of resistant TB strains, which support its alternative use over conventional DST in daily clinical practice. So, applying LPA would in turn enhance the proper MDR anti-tuberculous management with the proper drug regimen, increasing cure rate, decreasing rate of transmission, avoid redundant use of medication, thus providing good control of MDR-TB. This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted.

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