Molecular diagnosis of *Mycobacterium Tuberculosis*: A review Augustine Chijioke Udefi and Victor Olaosebikan

Federal Medical Center, Nigeria

Abstract:

There is an ongoing demand to shorten the turnaround time for accurate detection and monitoring of tuberculosis infection, which is an advantage the molecular methods possess over conventional methods. The objective was to review various molecular diagnostic methods and how it can be applied for the detection of this pathogen. A broad and thorough search through peer reviewed publications, conferences, articles, and a book was the method adopted for this review. It was found that molecular techniques aided the detection of antimicrobial resistance in addition to sensitive and specific detection of the pathogen, which leads to reduction in morbidity and mortality due to lesser complications attributed to delayed turnaround time.

Introduction:

Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is an infectious disease that poses a major global public health problem for both developing and developed countries. The World Health Organization (WHO) estimates that in 2015, 1.8 million people died from TB (including 0.4 million who were HIV-positive) (World Health Organization, 2016).

In the same year, quite 95% of TB deaths occurred in low- and middle-income countries, and 170,000 children died of TB (excluding children with HIV). the standard site of TB infection is that the lungs (pulmonary TB), but other organ systems are often involved (extrapulmonary TB) in spreading *M. tuberculosis*, including: pleural, lymphatic, urogenital, osteoarticular. The frequency of extrapulmonary disease increases with immune deficiency states, like acquired immune deficiency syndrome patients (in whom extrapulmonary disease accounts for 50–60%), or by the dissemination of *M. tuberculosis* throughout multiple organ systems (Miliary TB). Rapid and early diagnosis of TB and initiating optimal treatment wouldn't only enable a cure of a private patient but will reduce future numbers of TB cases.

Advancements in molecular process for MTB detection has reduces the time of diagnosis to a few days, whereas diagnosis by conventional culture systems needs several weeks (Cho et al., 2015). The most of the of molecular tests have been aimed at the detection of MTB specific nucleic acids, both DNA and RNA, by using amplification methods such as polymerase chain reaction (PCR), and detection of genes mutation that are related with the resistance to anti-TB drugs by sequencing or nucleic acid hybridization (Balasingham et al., 2009).

Common Molecular Methods for Diagnosis of M. tuberculosis

1. The Xpert MTB/RIF assay: This is a nuclear acid amplification-based test using a cartridge based on the Gene-Xpert Instrument System (CDC, 2013). The basis of the Xpert MTB/RIF assay is a real-time PCR that can be used to detect DNA sequences specific to the MTB in sputum samples (Friedrich, 2013). The Xpert MTB/RIF assay detects the rifampicin resistance by PCR amplification of the 81bp fragment of the MTB rpoB gene and subsequent probing of this region for rifampicin resistantassociated mutations and the results can be obtained within 2 hours (Boehme, 2010).



2. Loop-Mediated Isothermal Amplification: This method amplifies very few copies of acheivements DNA with high specificity, efficiency, and rapidity under isothermal conditions using a set of 4 specially designed primers and a DNA polymerase with strand displacement activity (Bentaleb et al., 2016).



- 3. **DNA Probes:** The tests are based on species-specific DNA probes that hybridize with rRNA released from the bacteria. The probes have been evaluated extensively in clinical practice and are rapid, sensitive, and specific (Lebrun et al., 1992). Moreover, probes are not available for all the pathogenic mycobacterial species and those isolates must be identified by other methods.
- 4. Line Probe Assay: Line probe assay Genotype MTBDRplus assay is validated for both direct use on smear-positive pulmonary specimens and on isolates of Mycobacterium tuberculosis grown on liquid medium or in solid medium. The assay is based on multiplex polymerase chain reaction (PCR) combined with reverse hybridization on nitrocellulose strips targeting common mutations for RIF and INH resistance (Sharma et al., 2014).

5. Whole Genome Sequencing (WGS): The method utilizes MTB DNA-specific biotinylated RNA baits to capture full MTB genomes directly from non-cultured sputum samples (Brown et al., 2015). WGS is becoming an affordable and accessible method that can identify microevolution within MTB lineages as they are transmitted between hosts (Walker et al., 2013). The WGS can detect various types of mutations better than the Xpert MTB assay. Moreover, WGS could avoid false positives when a polymorphism in the rifampicin-resistance determining region (RRDR) of rpoB is detected (Witney et al., 2016).

Conclusion:

Molecular techniques are revolutionizing the world of diagnostic medicine, and understanding their strengths and weaknesses would enable us better understand M. tuberculosis as well.

Recommendations:

The molecular diagnostic testing for active pulmonary TB is a promising prospect and should be fortified in a way that could improve population health.