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False Positive Rifampicin Resistant Report with Xpert Mtb/Rif Assay in Sputum Samples with Bacterial Grade of Low and Very Low: A Case Report

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Abstract

Xpert *MTB*/RIF assay is a molecular technique which detects *Mycobacterium tuberculosis* (*MTB*) and rifampicin resistance simultaneously in two hours. Based on the probes cycle threshold (Ct), the assay provides a semi-quantitative *MTB* detection which is the number of polymerase chain reaction (PCR) cycles required to amplify *MTB* deoxyribonucleic acid (DNA) to a level which can be detected. *MTB* detection is reported as High, Medium, Low or Very Low, while rifampicin resistance is reported as detected, not detected or indeterminate. Rifampicin resistant results with low or very low *MTB* detection grade or indeterminate rifampicin resistant results should be confirmed with a gold standard culture based drug susceptibility testing (DST).

Key Words: Mycobacterium Tuberculosis; Rifampicin; Tuberculosis; Xpert; Cycle Threshold.

1. Introduction

Gene-Xpert MTB/RIF assay is a molecular test which is automated and cartridge based, it detects presence of *Mycobacterium tuberculosis (MTB)* and rifampicin resistance simultaneously [1].

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The assay employs real time polymerase chain reaction (PCR).The reagent for DNA extraction, PCR amplification, internal controls and the five partially overlapping fluorescent probes A to E which target the rifampicin resistance determining region of *MTB* rpoB gene are all contained in a single use cartridge[1].

Based on the probes cycle threshold (Ct), the assay provides a semi-quantitative *MTB* detection which is the number of PCR cycles required to amplify *MTB* DNA to a level which can be detected [2]. The results for *MTB* detection is reported as High (Ct< 16), Medium (Ct 16-22), Low (Ct 22-28) or Very low (Ct >28) [2]. In samples where rifampicin is susceptible all the five probes exactly match to the PCR amplified *MTB* DNA and their Ct values are the same [2-3]. When there are mutations in the rpoB gene the hybridization dynamics change between the amplicon and the probes, this causes a difference between the Ct values of the probes [3].

Rifampicin resistant results with low or very low *MTB* detection grade should be confirmed with a gold standard culture based drug susceptibility testing (DST) [4].

2. Case Study

A 32 year old Kenyan Man consulted a physician for persistent cough of two weeks that was associated with night sweats, fatigue, marked weight loss and fevers. His serological status for HIV was positive and hepatitis B surface antigen negative, the full blood count was normal and had no history of TB treatment previously. Vital signs on examination revealed normal blood pressure, pulse, with high fever. Gene-Xpert *MTB*/RIF assay on sputum revealed *MTB* detected low, rifampicin resistance indeterminate. A repeat test to confirm the rifampicin status using an early morning sample revealed *MTB* detected low, rifampicin resistance detected. Sputum sample was collected and sent for culture and drug susceptibility. In the meantime the patient was commenced on first line tuberculosis treatment awaiting culture results. The regimen consisted of rifampicin(R) (10mg/kg/day), isoniazid (H) (5mg /kg/day), pyrazinamide (Z) (30mg/kg/day), and ethambutol (E) (20mg/kg /day). The first two months consisted of (RHZE), with the last four consisting of (RH). The culture and drug susceptibility reports obtained showed growth of mycobacteria which was susceptible to rifampicin, isoniazid, ethambutol and pyrazinamide. After 6months of treatment the patient totally recovered.

3. Discussion

In our patient as in many cases which are HIV positive, the bacillary burden in sputum was low, sputum results on the first sample revealed *MTB* detected low; rif resistance indeterminate while the second sample was *MTB* detected low; rif resistance detected. Both samples had a low bacillary load, which is a feature mostly encountered in HIV positive individuals [5-6]. Our patient was HIV positive; hence therefore the low bacillary load would be attributed to the positive HIV status. As in many cases described previously of low bacillary load and the inconclusive rifampicin susceptibility results the treatment was delayed for two more days for a repeat of the indeterminate rifampicin test result. The sample quality was appropriate and symptoms of pulmonary tuberculosis were present. No history of exposure to a patient treated for drug susceptible or resistant tuberculosis. Bacteriological confirmation was through phenotypic culture and drug susceptibility, which confirmed *mycobacterium tuberculosis* sensitive to streptomycin, isoniazid, rifampicin, ethambutol and

pyrazinamide. Other false positive rifampicin results have been reported, Marlowe and his colleagues identified a specimen that was repeatedly rifampicin resistant on Xpert but susceptible on phenotypic DST [7]. Also Theron and his colleagues identified six rifampicin resistant cases on Xpert, five of which were susceptible on phenotypic DST [8]. Culture and phenotypic drug susceptibility testing remains essential, especially in drug susceptibility tests involving nucleic acid amplification tests where there are inconclusive results [6].

4. Recommendations

The health care personnel need to have in depth knowledge about test performance and interpretation of results; also culture and phenotypic drug susceptibility testing should be accessible.

5. Limitation

The results of this report can't be generalized to the wider population

6. Conclusion

In conclusion our case emphasizes the importance of culture and phenotypic drug susceptibility testing in susceptibility tests involving nucleic acid amplification tests; this is important especially in cases of inconclusive results i.e. indeterminate rifampicin test results. Rifampicin resistant diagnosis in tests with very low and low *MTB* detection grade should be confirmed with a gold standard culture based DST**.**

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