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Detection of Pre-extensively Drug Resistance (PRE-XDR TB) and Extensively Drug Resistance (XDR-TB) among Pulmonary Multidrug Resistant Tuberculosis (MDR-TB) Patient by Line Probe Assay

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A B S T R A C T

The rate of multidrug-resistant (MDR) and extensively drug-resistant (XDR) tuberculosis (TB) has been steadily increasing in developing countries like India. The availability of rapid and reliable methods for the detection of drug resistance to second-line drugs is vital for adequate patient management. MTBDRsl-2 is newer DNA based test recommended by WHO for screening of XDR and Pre XDR patients.MTBDRsl-2 is a rapid, screening method that detects genetic mutation responsible for resistance in second line injectable drugs in tuberculous patients mainly against Flouroquinolones and /or second line injectable drugs. MTBDRsl-2 produces results in just 24-48 hours, as compared to culture methods that takes 3 months or longer. It helps to physician to make line of treatment for confirmed rifampicin resistant or MDR-TB patients into either the shorter MDR-TB regimen or the conventional regimen. This test is the first and only WHO recommended rapid test for detection of additional resistance in MDR-TB patients as well as XDR-TB. Confirmation of Pre XDR and XDR status of patient has to be done by liquid culture DST which requires 1-2 months. As it is rapid it can be helpful in Known MDR patients for further line of management. Total 700 samples are enrolled in the study at Culture and DST Laboratory, Jamanagar Gujrat during Apriln19 to June 19. Only MDR or RR confirmed sputum samples or cultures are tested for second line LPA Version 2. Samples were processed as per standard protocols for Line Probe Assay as per kit instruction. Total 700 MDR Sputum/Culture Samples were tested, out of these 143(20.42%) pre XDR (resistant to either Floroquionolones or one of injectable second line) and 23(3.2%) XDR were found. MDRSl version 2 is rapid, reliable, and accurate Screening method for the identification and detection of Second line drug resistance in M. tuberculosis strains. Early detection of Pre XDR and XDR status helps to manage the tuberculosis patient by helping in deciding line of treatment.

Introduction

Pre-extensively drug resistant tuberculosis (pre-XDR-TB) is a comparatively new term and is defined as TB with resistance to rifampicin (RMP) and isoniazide (INH) with additional resistance to either a FLQ (Fluoroquinolone) or ISL (Injectable second

line) agent but not against both these drugs simultaneously. XDR-TB is defined as TB resistant to RMP and INH (MDR-TB) with additional resistance to second line anti-TB drugs i.e. to any FLQs, and to at least one of the three injectable second-line drugs (ISL) naming amikacin, kanamycine and capreomycin. The prevalence of XDR-TB is 9.5% worldwide. Treatment of XDR-TB is complicated, as it requires the use of second-line drugs that are less effective and more toxic, thus demanding longer treatment duration. Detection of pre-XDR-TB cases among MDR-TB patients is an important step in the prevention of treatment failure of MDR-TB and in addition, it helps to take appropriate measures to halt the progression towards XDR-TB.

Pre XDR and XDR tuberculosis (TB) has become a serious threat to global TB control due to difficulties in Early diagnosis and treatment**,** and the associated high costs.[(1)]. Early detection of people with MDR-TB is one of the major bottlenecks in tackling this epidemic. Of the 480 000 MDR-TB cases estimated to have occurred in 2014, only about a quarter – 123 000 – were detected and reported to national authorities.[(2)]In May 2016, WHO issued new recommendations on the use of a rapid diagnostic test – a line probe assay to detect resistance to second-line anti-TB drugs (SL-LPA) (3). WHO recommends this rapid diagnostic test for identifying those MDR- or rifampicin-resistant TB patients who can be placed on the shorter MDR-TB regimen.[(4)] The results of this test will also be critical in placing patients on targeted conventional MDR-TB regimens with improved outcomes.

In India the rate of MDR and XDR TB infections has been steadily increasing[(19)] due to multiple incomplete treatment regimens and poor infection control practices. rapid, reliable, and accurate methods for the identification and detection of drug resistance in M. tuberculosis strains is necessary for adequate patient management, leading to improved outcomes, a reduction of infectiousness**,** [(8)]

Materials and Methods

Total 700 samples (Sputum or Culture isolate) of known MDR TB were included in this study during the period of April19 to June 19.Clinical specimens are processed using the NALC/NaOH method as per universal Guideline. The MTBDRsl test is based on the DNA•STRIP technology. The whole procedure is divided into three steps: (i) DNA extraction from decontaminated sputum specimens or cultured material (solid/liquid medium) – the necessary reagents are included in the kit, (ii) a multiplex amplification with biotinylated primers, and (iii) a reverse hybridization. All reagents needed for amplification, such as polymerase and primers, are included in the Amplification Mixes A and B (AM-A and AM-B) and are optimized for this test. The membrane strips are coated with specific probes complementary to the amplified nucleic acids. After chemical denaturation, the single stranded amplicons bind to the probes (hybridization). Highly specific binding of complementary DNA strands is ensured by stringent conditions which result from the combination of buffer composition and a certain temperature. Thus the probes reliably discriminate several sequence variations in the gene regions examined. The streptavidin-conjugated alkaline phosphatase binds to the amplicons' biotin via the streptavidin moiety. Finally, the alkaline phosphatise transforms an added substrate into a dye which becomes visible on the membrane strips as a colored precipitate. A template ensures the easy and fast interpretation of the banding pattern obtained.

Results and Discussion

Total 700 MDR sputum Samples were tested, out of these 143(20.42%) pre XDR (Resistant to either Floroquionolonesorone of injectable second line) and 23 (3.2%)s XDR were found.

Table.1 Resistance pattern of floroquinolones & second line injectable

Total No. of Samples	Resistance for Fluoroquinolones	Resistance for Second Line Injectable.	Resistance to both Fluoroquinolones & Second Line Injectable
700	131(18.71%)	12 (1.71%)	23(3.2%)

Table.2 Prevalence of Pre XDR and XDR

No. of Pre XDR (Resistance for either Fluoroquinolones or Second Line Injectable.)	No. of XDR Resistance to both Fluoroquinolones &Second Line Injectable	Total No. of Resistance seen
143(20.42%)	23 (3.2%)s	166(23.7%)

MTBDRsl –2 is a DNA-based test that identifies genetic mutations in MDR-TB strains, making them resistant to fluoroquinolones and injectable second-line TB drugs. This test is the first and only WHO recommended rapid test for detection of additional resistance in MDR-TB patients as well as XDR-TB. The SL-LPA produces results in just 24-48 hours, as compare 3 months taken by Culture. The Genotype MTBDRsl assay is relatively easy to introduce into routine settings. It only needs trained staff and basic infrastructures as required in PCR lab. As compared to liquid culture which takes 1-2 months for reporting, it gives result within 1 day. So it is advantageous to use MTBDRsl -2 as routine screening method for early detection of XDR and Pre XDR condition.

The prevalence rate of pre-XDR-TB among MDR-TB patients was reported to be 12.1 per cent in Poland, 16.7 per cent in Nigeria, 18 per cent in California, 31 per cent in China and 51.4 per cent in Philippines (31,32,33). Prevalence of XDR in our study is 20.32% relating to other studies. The actual incidence and prevalence of XDR-TB in India is not available. A few scattered reports reveal the prevalence ranging from 2.4-33. Study by Rajeskaran *et al.*, Chennai shows XDR prevalence of 1.6%, study of Ramchandran *et al.*, Gujrat shows 3.2% and study by Paramshivam *et al.*, Chennai shows prevalence of 4.2%. Our study shows XDR prevalence of 3.2% near about similar as in other part of country.

Early screening of Pre XDR conditions is helpful for patients of MDR status to choose the proper Antibiotic regimen to prevent any spread of infectiousness and preventing patient for going toward XDR conditions. In XDR patient it will be helpful for better patient management by choosing proper line of management preventing spread to community (Table 1 and 2).

In conclusions, MDRSl version 2 is rapid, reliable, and accurate Screening method for the identification and detection of Second line drug resistance in M. tuberculosis strains. Early detection of Pre XDR and XDR status helps to manage the tuberculosis patient by helping in deciding line of treatment. Liquid culture DST is always required for

confirmation of resistance in positive cases. As liquid culture takes long time, MDRsl -2 reduce the time for diagnosis of susceptible group and helping in reducing infectivity.

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