Early detection of multidrug resistant (MDR) *Mycobacterium tuberculosis* in a single tube with in-house designed fluorescence resonance energy transfer (FRET) probes using real-time PCR

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Rapid and correct diagnosis is crucial for the management of multidrug resistance (MDR) in *Mycobacterium tuberculosis* (MTB). The present study aims at rapid diagnosis for identification of multidrug resistance tuberculosis (MDR-TB) using real-time PCR. FRET hybridization probes targeting most prominent four selected codons for rpoB526 and 531 and for katG314 and 315 genes were designed and evaluated on 143 clinical MTB isolates and paired sputa for rapid detection of MDR-TB. The results of real-time PCR were compared with gold standard L-J proportion method and further validated by DNA sequencing. Of the 143 MTB positive cultures, 85 and 58 isolates were found to be 'MDR' and 'pan susceptible', respectively by proportion L-J method. The sensitivity of real-time PCR for the detection of rifampicin (RIF) and isoniazid (INH) were 85.88 and 94.11%, respectively, and the specificity of method was found to be 98.27%. DNA sequencing of 31 MTB isolates having distinct melting temperature (Tm) as compared to the standard drug susceptible H37Rv strain showed 100% concordance with real-time PCR results. DNA sequencing revealed the mutations at Ser531Leu, His526Asp of rpoB gene and Ser315Thr, Thr314Pro of katG gene in RIF and INH resistance cases. This real-time PCR assay that targets limited number of loci in a selected range ensures direct and rapid detection of MDR-TB in Indian settings. However, future studies for revalidation as well as refinement are required to break the limitations of MDR-TB detection.

Speaker Biography

Devendra Singh Chauhan is a scientist in the department of Microbiology and Molecular biology at National JALMA Institute for Leprosy & other Mycobacterial Diseases(ICMR). His interest and researches are towards investigation of *Mycobacterium Tuberculosis* and has successfully worked on the various results of the same. He also worked as a core scientist in group of Dr. V M Katoch, Former secretary DHR & DG, ICMR (Indian Council of Medical Research).

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