A multiplex PCR assay for *Mycobacterium tuberculosis* Beijing/W lineage identification

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Mycobacterium tuberculosis (MTB), causative agent of Tuberculosis (TB), reveals diverse biological and clinical properties linked to their lineages. For effective TB control, identifying the frequency of circulating strains in a country is important. Globally, seven MTB lineages have been identified and East-Asian Beijing/W lineage gained attention due to its wide-spread nature and links to drug resistance. In Sri Lanka, little is known about the lineage pattern; however, in 2003, 8.8% and in 2011, 14% were reported as Beijing strains. The present study was conducted to identify the occurrence of Beijing/W MTB strains among patients attending the general hospital, Kandy, using molecular methods.

Ziehl-Nielsen positive sputa were collected from first-time visit patients attending the Kandy hospital (period - 3 months). Sputa were processed with Modified Petroff`s method and cultured on Lowenstein-Jensen medium. DNA, extracted from culture isolates (n=17) were subjected to multiplex PCR using primers specific for Rv0627c gene. The gene possesses a single nucleotide polymorphism, C to G at position 426, unique to Beijing lineage. PCR was carried out in a 25 µl reaction mixture, containing 75ng of DNA, 0.33mM of each dNTP, 0.33µM of each Fw and R1 primers (Fw-GTCACTGAACGTGGCCGGCTC, R1-TCGGTCACCGTTTTTGTAGGTGACCGTC), 0.13µM of R2 primer (R2-AGCAACCTCGCAATCTGACC), 1xPCR buffer, 2.25mM $MgCl_2$ and 0.8units of Taq-DNA polymerase (Promega). Thermo-cycle program was set at initial denaturation at 95^0C for 1 min, followed by 35 cycles of denaturation at 95^0C for 10 s, annealing at 66^0C for 30 s elongation at 72^0C for 30s, and final extension at 72^0C for 3 min. A confirmed Beijing strain and H_{37}Rv were used as positive and negative controls respectively. Amplicons were run on a 2% agarose gel stained with ethidium-bromide.

Patients included in the study, were in the age group of 15-60 years and majority were males (n=15). Multiplex PCR assay identified 07 isolates as Beijing/W strains yield two bands 163 bp and 261 bp in contrast to non-Beijing strains which produced only the 261 bp band.

As 41% of the study population had strains belonged to the Beijing/W lineage, it is vital to perform Drug Sensitivity Testing to identify the lineage specific drug resistance.

Keywords: Beijing/W genotype, Multiplex PCR assay, Tuberculosis

Funding: *National science foundation (Grant number- NSF-PSF/ICRP/2017/HS/01)*