Type: Poster Presentation

Final Abstract Number: 43.118
Session: Poster Session III
Date: Saturday, March 5, 2016
Time: 12:45-14:15
Room: Hall 3 (Posters & Exhibition)

Insertion Sequence IS6110 mapping, a tool to characterise TB strains into genetic lineages

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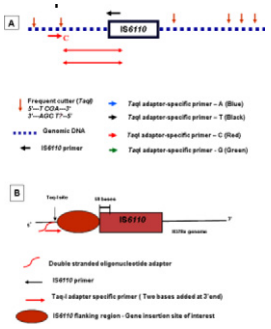
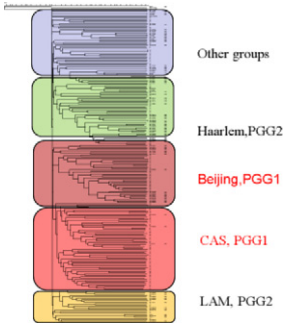
Background: Tuberculosis (TB) along with HIV infection is the major cause of mortality worldwide including the 29 million people in Nepal. The steady rise in the number of Multi-drug Resistant (MDR) TB cases in the last few years has increased the challenges facing the scientists and health professionals alike. With no previous epidemiological data available on transmission patterns of *Mycobacterium tuberculosis* complex (MTBC) in Nepal, the focus of this study is to categorise the TB samples for the first time using IS*6110* fluorescent amplified fragment length polymorphism (FAFLP) PCR into different genetic lineages.

Methods & Materials: The bacterial DNA from clinical isolates of 176 TB patients in Nepal along with the reference strain H37Rv were extracted using the CTAB method and subjected to FAFLP PCR, using four differentially labelled selective primers. The samples separated on the ABI Genetic Analyser 3730xl were then analysed using the PeakScanner software and were identified using their fluorescent tag. The 4-dye FAFLP data collected from the different profiles were later recorded in the BioNumerics software v6.1and compared with the reference global collection of TB samples.



Method schematic of 4-dye IS6110 FAFLP PCR. In the example shown above (A), red fragment is generated as TaqI -C anneals to the C base in the DNA where base C is amplified and in example (B) exact insertion of IS6110 in the Mtb genome is identified using

Results: Out of 176 samples analysed, 64 samples belong to the Central Asian (CAS) lineage or principal genetic group 1(PGG1), 33 samples belong to the Beijing lineage (PGG1) and the rest of the samples belong to other genetic groups – LAM, Haarlem, X (PGG2) and T (PGG3). Also, all but two of the sixteen insertion sites of H37Rv were mapped using this technique.



UPGMA derived Dendrogram showing the distribution of the 176 bacterial DNA isolates with CAS and Beijing (shown in red) lineages (>50%) being the major principal genetic group in Nepal

Conclusion: From the data above, it is clear that 55% of the samples fall under the CAS and the Beijing group. This novel information on TB population in Nepal is geographically relevant as it is surrounded by China in the north (dominated by Beijing strains) and the other Central Asian countries in the south (dominated by CAS strains). As the prevalence of TB infection including the MDR types is high in the Nepalese population, the 4-dye FAFLP typing technique will not only aid the contact tracing of samples but also shows a picture on the predominant PGGs found in Nepal which can be helpful in future epidemiological surveillance or outbreaks.

http://dx.doi.org/10.1016/j.ijid.2016.02.856