Insights into the genetic variability of LipL32 and Loa22 among 25 Sri Lankan *Leptospira* strains

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Introduction and Objectives: During leptospiral infection, the host innate immune response is initiated through recognition of pathogen-associated molecular patterns (PAMPs) by Toll-like receptor 2 (TLR2). LipL32 and Loa22, were identified as two major outer membrane receptors that interact with host TLR2. This study aimed to investigate these molecules' evolutionary conservation and genetic variability in recently isolated *Leptospira* strains from clinical samples.

Methods: We retrieved whole-genome sequences (WGS) of 25 Sri Lankan *Leptospira* isolates (previously published by our group; prefix FMAS_) and 15 reference strains (pathogenic-13,inter-pathogenic-1, non-pathogenic-1) from NCBI. All 40 WGS were annotated using Rapid Annotation Subsystem Technology (RAST). Protein BLAST was performed on RAST comparative tool to select the best matching sequences (based on bit score, E-value, identity) among already published LipL32(1) and Loa22(15) with all 40 WGS. The best matching gene sequences were used to extract the gene/protein sequences from annotated 40 WGS on RAST. Multiple Sequence Alignment was performed implementing MUSCLE followed by phylogenetic analyses on MEGA 11 using Maximum Likelihood (1000 bootstrap). SNP analysis and Insertion Sequence detection were performed on BVBRC and IS Finder database respectively.

Results: LipL32 (819 bp/ 272 aa) and Loa22 (588bp/195aa) gene sequences of 25 Sri Lankan *Leptospira* were published on GenBank with the Accession numbers of PV471625, PV539477-PV539525. Phylogenetic trees showed less divergence of LipL32 and Loa22 among 25 Sri Lankan *Leptospira* isolates. Pathogenic isolates, *L.interrogans* (15), *L.kirschneri* (1), *L.borgpetersenii* (7), and *L.weilii* (2) were clustered in one separate clade, which outgroups non-pathogenic *L.biflexa* Patoc I, while intermediate pathogenic *L.licerasiae* clustered with *L.weilii*. All Loa22 gene sequences of 25 isolates grouped into one major arm aligned with pathogenic references. Among 25 isolates *L.weilii* strains FMAS_PD2, RT1 showed LipL32 protein polymorphism Lys to Arg at183 aa. *L.kirschneri* strains FMAS_PN5 showed Loa22 protein polymorphism Lys to Arg at 180 aa. Lys to Arg may affect the protein interaction and binding affinity due to the properties of Guanidine group of Arginine.

Conclusion: Our findings highlight the high conservation of LipL32 and Loa22, reflecting their importance in pathogenesis and reinforcing their potential as stable targets for molecular diagnostics and serological assays.

***Keywords*:** *Leptospira, LipL32, Loa22, Sri Lanka*

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