Detection of Variably Present Genotypes, *bimA$_{BP}$/bimA$_{BM}$, fhaB3* YLF/BTFC and *LPSA* in Sri Lankan Clinical Isolates of *Burkholderia pseudomallei* Using Real-Time PCR Based Molecular Assay

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Burkholderia pseudomallei is the causative agent of a potentially fatal disease, melioidosis, with clinical presentations such as pneumonia, skin complications, neurological complications and sepsis. The bacterium possesses several variably present genes such as *B. pseudomallei* intracellular motility factor *BimA (bimA$_{BP//}$BimA$_{BM}$)*, filamentous hemagglutinin B (fhaB3), Yersinia like fimbrial/ *Burkholderia thailandensis* flagellum chemotaxis (YLF/BTFC) gene clusters and Lipopolysaccharide O antigen type A (LPSA). These genes have been reported to be differentially associated with bacterial survival in the host cells and virulence. The aim of this study was to determine the prevalence of *bimA$_{BP}$/bimA$_{BM}$, fhaB3, YLF/BTFC*, and LPSA genotypes in 51 clinical isolates of *B. pseudomallei* in Sri Lanka. Total genomic bacterial DNA extracted from 51 clinical culture positive isolates confirmed by lpxO (Lipid A Hydroxylase) was tested for its concentration using agarose gel electrophoresis. Genotyping was performed using fluorescent dye-based RT- PCR molecular assays with oligonucleotide primers targeting each gene specifically. Prevalence of *fhab3, YLF, bimA$_{BP}$, bimA$_{BM}$* and LPSA were found to be 50.98% (n=26), 92.16% (n=47), 68.63% (n=35), 23.53% (n=12) and 68.63% (n=35) respectively. High genetic diversity was observed among clinical isolates and the study population is of mixed type. The prevalence of isolates with BTFC was 7.84%, similar to the Australian *B. pseudomallei* population whereas the prevalence of YLF in Thailand is 100%. Within the study population, the mortality rate was 47.06%. Diabetes and alcoholism were found as the major risk factors among other risk factors like kidney failure, asthma, and Cushing's syndrome.

Keywords*: Melioidosis, Genotyping, Burkholderia pseudomallei, Sri Lanka, YLF/BTFC, clinical isolates, risk factors, real-time PCR*