Phylogeny of Mycobacterium ulcerans strains in Nigeria - A Preliminary Study

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Abstract

Buruli ulcer (BU), caused by Mycobacterium ulcerans, is a necrotizing disease of the skin. The application of bacterial population genomics to the recent studies of Mycobacterium ulcerans gave increased knowledge of its transmission, epidemiology and evolution. PCR techniques were deployed to confirm BU among suspected cases from five states of southwest, Nigeria. Some of the Mycobacterium ulcerans IS2404-positive samples were subjected to Sanger sequencing followed by BLASTs and phylogenetic analysis. All the organisms were identified as M. ulcerans through BLASTs. The phylogenetic tree showed genetic similarity to SGL03 and Ag99 strains.

Results

Following BLASTn analysis, all the samples were identified as Mycobacterium ulcerans. The phylogenetic tree showed that the identified strains were more genetically identical (96%) to SGL03 strain from the Democratic Republic of Congo (DRC) with only one showing evolutionary similarity to Agy99, a reference strain from Ghana than to M. ulcerans subsp. shinshinense a reference strain from Japan.

Discussion

This M. ulcerans strain diversity has not been reported from Nigeria before. This work represents an advance in biomedical science in that it has not only now bridged the research information gap that has existed in the country for over 44 years but has also opened up a vista of research opportunities for further work on Buruli ulcer in Nigeria. Being the first report, it is expected to facilitate further genomic studies of the pathogen towards better understanding of its epidemiological profile.

Acknowledgement

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Methods and Materials

PCR techniques were deployed to confirm BU among suspected cases from five states of south-west, Nigeria. This study examined the phylogeny of M. ulcerans strains from some of the BU-positive samples in order to evaluate the phylogenetic relatedness of the Mycobacterium ulcerans strains sequenced in this study to other referenced strains. Sanger sequencing of the samples was followed by BLASTs and phylogenetic analysis (Jukes-Cantor model). A consensus DNA sequence contig was generated from the forward and reverse sequences with manual base calling carried out for regions of ambiguities.

For the comparative genomic analysis, reference genomes of M. ulcerans (Accession Numbers: LR135168 and CP000325), M. ulcerans subspecies shinshinense (Accession Numbers: AP017624 and AP017635) and M. ulcerans IS2404 gene (Accession Numbers: KM459600 and KM459601) were obtained from the NCBI database. These sequences were then aligned with the sequences from this study using MAFFT v7.402 (Katoh et al., 2002). Using GeneiousPrime, an approximately 2199bp of the IS2404 region of the genome which aligned best with the sequences from this study was extracted and used to infer a maximum likelihood tree using IQTREE v1.6.3 (Nguyen et al., 2015). ModelFinder (Kalyaanamoorthy et al., 2017) selected TIM31+F as the best-fit model according to Bayesian Information Criterion (BIC). FigTree v1.4.4 (http://tree.bio.ed.ac.uk/software/figtree/) was used to view and edit the tree (Fig 2).

Conclusion

This M. ulcerans strain diversity has not been reported from Nigeria before. This work represents an advance in biomedical science in that it has not only now bridged the research information gap that has existed in the country for over 44 years but has also opened up a vista of research opportunities for further work on Buruli ulcer in Nigeria. Being the first report, it is expected to facilitate further genomic studies of the pathogen towards better understanding of its epidemiological profile.

Selected References