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* IS*2404*-positive samples were subjected to Sanger sequencing followed by BLASTn and phylogenetic analysis. All the organisms were identified as *M.ulcerans* through BLASTn. The phylogenetic tree showed genetic similarity to SGL03 and Agy99 strains. The study represents a pivotal strategy towards genomic profiling of *Mycobacterium ulcerans* strains, which has not hitherto been studied in the country.

Keywords: Phylogeny, Buruli ulcer, microbial population genomics, *Mycobacterium ulcerans*, south-west, Nigeria

Introduction

Buruli ulcer, an emerging, neglected tropical disease caused by *Mycobacterium ulcerans* (an environmental mycobacterium) is a chronic necrotizing disease of the skin and soft tissue that causes disabling symptoms and consequences, as well as a high burden of morbidity, shame, and economic implications for patients and their families (Oke *et al*., 2018). Although the mode of transmission of *Mycobacterium ulcerans* is unknown or poorly understood, residing near potentially polluted water sources has been recognized as a major risk factor for Buruli ulcer. However, the disease can also be acquired without wetland exposure (Muleta *et al*., 2021).. Mycolactone, a cytotoxic macrolide lipid produced by *Mycobacterium ulcerans*, is the key virulence determinant(Röltgen *et al*., 2020).

BU, as the third most common mycobacteriosis after tuberculosis and leprosy has been documented in at least 33 countries, with the highest prevalence in West Africa and Australia. In 1998, World Health Organization (WHO) regarded Buruli ulcer as an emerging infectious disease

The clinical characteristics of Buruli ulcer and its physiopathology are becoming well understood, although little is known regarding genetic diversity of the causative pathogen especially in Nigeria.. For a disease with incomplete knowledge regarding its reservoir and transmission the quest to study the genomic profile of its causative agent is quite essential to unravel facts that will lead to the in-depth understanding of its epidemiology which makes the gene sequencing of *M. ulcerans* an attractive exercise. The application of genomics to investigate *Mycobacterium ulcerans* diversity has helped us to have some insights about its evolution (Vandelannoote *et al*., 2014). By genetic analyses, *Mycobacterium marinum*, an aquatic mycobacterium was discovered as the progenitor (the ancestor) of *M. ulcerans* (Stinear and Johnson, 2007).

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 BLASTn and phylogenetic analysis (Jukes-Canter 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 shinshuense* (Accession Numbers: AP017624 and AP017635) and *M. ulcerans* IS*2404* 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 219bp of the IS*2404* 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 TPM3u+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).

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 shinshuense* a reference strain from Japan.

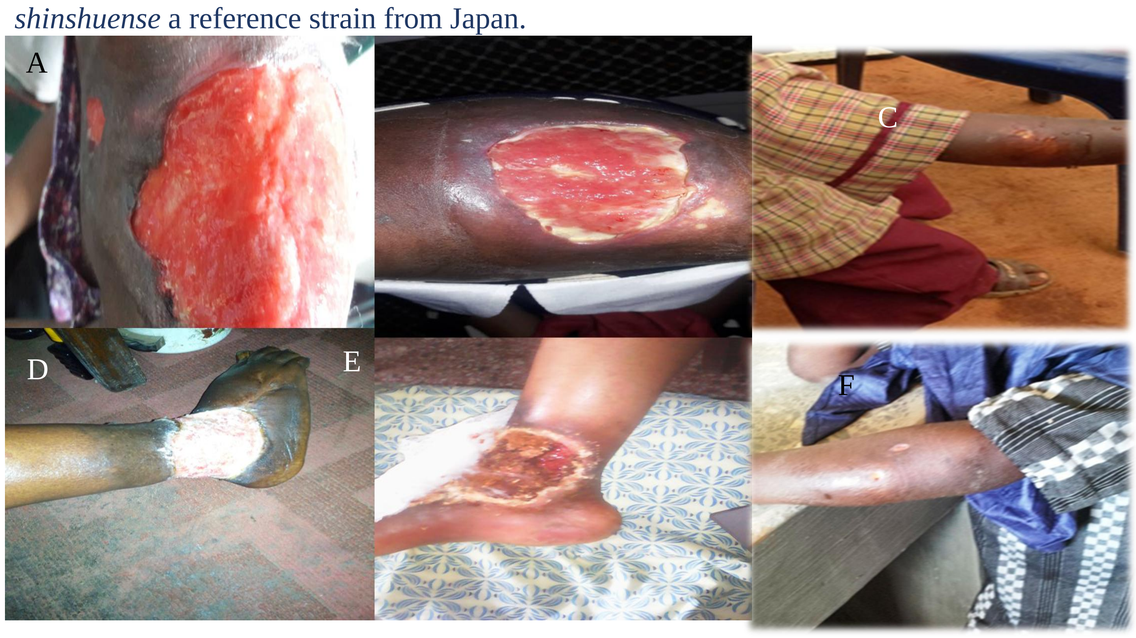

Figure 1| Images of selected Buruli ulcer lesions obtained from the some participants in this study

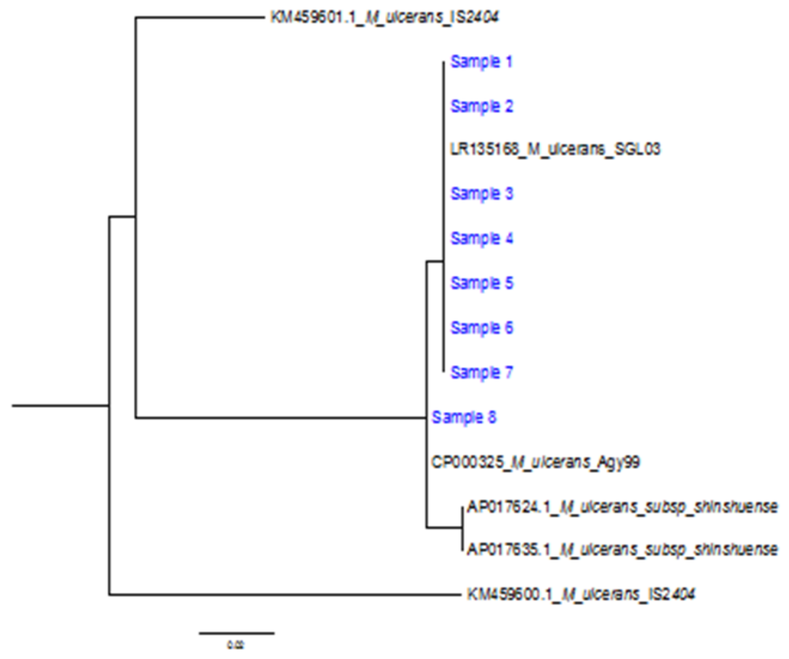

Figure 2| Genetic relationship among the *M. ulcerans* isolates from this study (coloured blue) and between six (6) other *M. ulcerans* sequences obtained from the NCBI database. A maximum-likelihood consensus phylogeny was inferred.

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.

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