

RESEARCH ARTICLE

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IS2404-Based Genomic Profiling of *Mycobacterium ulcerans* strains in Southwest, Nigeria: A Preliminary Study

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Abstract

Objectives: Buruli ulcer (BU), caused by *Mycobacterium ulcerans* (MU), is a necrotising skin disease which has been reported in over 33 countries. The application of bacterial population genomics to the studies of MU has brought about increased knowledge about its evolution and epidemiology.

Method: Nested PCR techniques were deployed to confirm BU among 256 suspected cases from five states in Southwest Nigeria. 157 (61.3%) of the samples were PCR positive for IS2404 of *M. ulcerans*. Eight (8) randomly selected IS2404-positive samples were subjected to Sanger sequencing due to resource constraints.

Results: All the organisms were identified as *M. ulcerans* through BLASTn, and the phylogenetic tree showed genetic similarity (>96%) to SGL03, a reference strain from the Democratic Republic of Congo.

Conclusion: This study provides the first IS2404-based genomic profiling of *Mycobacterium ulcerans* in Nigeria, addressing a critical epidemiological research gap.

Keywords: Genomic profiling, Buruli ulcer, *Mycobacterium ulcerans*, Southwest Nigeria, IS2404, Sanger sequencing

Plain English summary

A preliminary phylogenetic analysis of strains of *Mycobacterium ulcerans*, a causative agent of Buruli ulcer, was carried out in this study as the first of its kind in southwest Nigeria, revealing genetic similarities with reference strain SGL03, from the Democratic Republic of Congo.

Background

Buruli ulcer (BU), caused by *Mycobacterium ulcerans*, is a chronic skin disease in humans, characteristically presenting as a painless or, at best, minimally painful, slowly progressive, yet brutally disfiguring and crippling disease (1).

This neglected tropical disease, regarded as the third most common mycobacterial disease of humans, after tuberculosis and leprosy, and which primarily affects people in remote and rural areas, especially those living around wetland areas with stagnant lakes or slow-flowing streams, has been

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reported in over 33 countries, mainly tropical and subtropical regions of the world (2, 3). West Africa is now the epicentre of the disease (2).

Though its mode of transmission is unknown, the virulence of *Mycobacterium ulcerans* is mainly attributed to mycolactone, a cytotoxic toxin responsible for the necrosis associated with the disease (4, 5).

Historically, the first occurrence of BU was reported in 1967 in the Middle Belt Region of Nigeria and in 1975 (6), 24 cases were reported within and around the Ibadan metropolis in Southwest Nigeria (7). Thereafter, and for over 20 years, there was no research on BU in Nigeria. In 2006, WHO Buruli ulcer experts were able to document the presence of the disease in five Southeast and South-south states of Nigeria (8, 9).

The status of BU in Southwest Nigeria remained largely unknown until recently, when index cases of the disease were reported (2) in three states of Southwest Nigeria, aside from reports from outside the country (9, 10), indicative of its presence in the region.

The application of genomics to investigate *M. ulcerans* diversity has helped to provide detailed insights about its evolution (11). The Agy99 strain, the first fully sequenced *M. ulcerans* genome from Ghana (1999), serves as a reference for global comparisons. Meanwhile, whole genome sequencing of *M. ulcerans* isolates has been carried out in the Democratic Republic of Congo, Côte d'Ivoire, Togo, Benin Republic, Ghana and the Central African Republic (12, 13), but no such study has been done in Nigeria.

Methods

In this study, swabs and Fine Needle Aspiration samples (256) collected from patients with suspected-BU ulcerative and non-ulcerative lesions, respectively, in five states of Southwest, Nigeria (14), were subjected to Nested PCR targeted at IS2404 involving 25 pmol of each primer. The nested PCR targeted IS2404 using primers pGp1/pGp2 (stage 1) and pGp3/pGp4 (stage 2), with 40 and 35 cycles of the protocol, respectively, as previously described (15) to confirm BU among the suspected cases. The thermocycling profile was as follows: denaturation at 95°C for 15 min; 40 /35 cycles of 94°C for 30 min, 64°C for 1 min, and 72°C for 1 min 30 seconds; and a final extension at 72°C for 10 min. Throughout the cycling time, the heated lid function was used at 105°C.

Gene Sequencing (Organism Identification) and Phylogenetic Analysis

Amplicons (Nested PCR products) of 8 randomly selected among the BU-positive samples (157) were analysed on the Sanger sequencer ABI 3500XL platform, generating sequenced results for the samples. The integrity of the PCR amplicons was first visualised on a 1% agarose gel (CSL-AG500, Cleaver Scientific Ltd) stained with EZ-vision® Bluelight DNA Dye. Sequencing was then carried out with the Applied Biosystems™ BigDye™ Terminator v3.1 Cycle Sequencing Kit according to the manufacturer's manual. The labelled products were then cleaned with the ZR-96 DNA Sequencing Clean-up Kit, and the cleaned products were thereafter injected on Applied Biosystems ABI 3500XL Genetic Analyser with a 50cm array, using POP7. The generated sequences were submitted to GenBank (ID-2207789), and Accession Numbers (MK764762 - MK764777) were obtained.

The chromatograms of the sequences were viewed using Geneious Prime (v2019.2.3) software (16). A consensus DNA sequence contig was generated from the forward and reverse sequences, with manual base calling carried out for regions of ambiguity, and the nucleotide sequences were then subjected to BLASTn (Basic Local Alignment Search Tool) analysis.

For comparative genomic analysis, six reference sequences of *M. ulcerans* (Accession Numbers: LR135168 and CP000325); *M. ulcerans subspecies shinshuense* (Accession Numbers: AP017624 and AP017635); *M. ulcerans* IS2404 gene (Accession Numbers: KM459600 and KM459601) obtained from the NCBI database, were aligned with the eight sequences from this study using MAFFT v7.402 (17).

An approximately 219bp 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 (18) in the construction of a phylogeny. ModelFinder (19) selected TPM3u+F as the best-fit model according to the Bayesian Information Criterion (BIC). FigTree v1.4.4 (20) was used to view and edit the tree.

Results

From the phylogenetic tree (Fig. 1), all sequences from this study are genetically identical as they clustered closely together within the same Clade, implying that the genomic profile of *M. ulcerans* strains in Southwest Nigeria may be homogenous. All sequences were highly similar (>96% identity)

to the SGL03 strain from the Democratic Republic of Congo (DRC) than to the Agy99 strain from

Ghana and *M. ulcerans subsp shinshuense*, a reference strain from Japan.

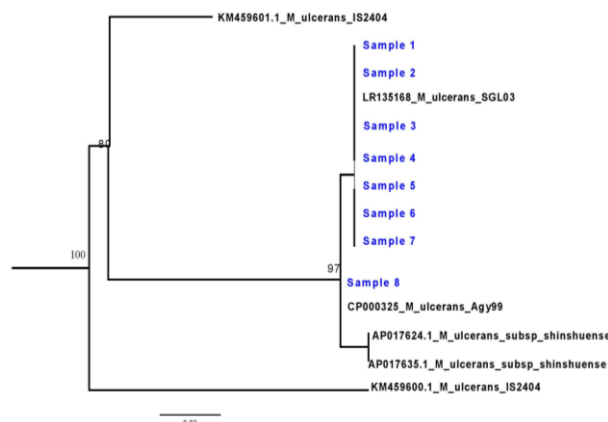


Figure 1: Phylogenetic relationship between the sequences from this study (coloured blue) and six other *M. ulcerans* sequences obtained from the NCBI database. (A) In blue colour (Sample 1 - Sample 8) are the eight randomly selected IS2404-positive samples. (B). In black are six reference sequences of *M. ulcerans*. (C)80, 97, 100 are bootstrap values

Discussion

Briand *et al* (2024) stated that although isolates from Africa are relatively homogenous, their study showed heterogeneity between some strains, with isolates from West Africa and Central Africa having identical genomic profiles (21). The close relatedness of the *M. ulcerans* strains to the DRC strain could be explained by the conclusion of Ablrdey *et al* (2015) that *M. ulcerans* can be introduced or imported into a region and then spread extensively (12). Unlike Briand *et al.* (2024), who reported heterogeneity among some African ulcerans isolates, this study found Nigerian strains to be genetically homogenous, with a high genetic similarity to the SGL03 reference strain from the Democratic Republic of Congo and much less so with the *M. ulcerans* Agy99 reference strain isolated in Ghana and the *M. ulcerans subsp shinshuense* strain from Japan. This similarity may reflect shared environmental reservoirs or historical introduction events, warranting further study.

Few works on *M. ulcerans* strain diversity and its genomic profiling have been reported in Nigeria before. This work represents an advance in biomedical science in that it has opened a vista of research opportunities for further work on Buruli ulcer in Nigeria. It is expected to facilitate further genomic studies of the pathogen towards a better understanding of its epidemiological profile and also help the healthcare stakeholders to adopt intervention strategies for the prevention and

control of BU in Southwest, Nigeria. The genetic similarity with strains from other countries is an indication of possible transport from such source countries. This gives room for more epidemiological studies for this disease, which has no exact known mode of transmission.

Conclusion

This study provides the first IS2404-based genomic profile of *M. ulcerans* in Southwest Nigeria, revealing high genetic similarity to DRC's SGL03 strain. These findings lay the foundation for expanded genomic and epidemiological studies to elucidate transmission pathways and inform targeted interventions, such as enhanced diagnostics and community surveillance. More extensive studies, with a bigger sample size, that will cover the entire Southern parts of Nigeria, are recommended.

List of abbreviations

BU: Buruli ulcer
 MU: *Mycobacterium ulcerans*
M. Ulcerans: *Mycobacterium ulcerans*
 PCR: Polymerase Chain Reaction

Declarations

Ethics approval and consent to participate

Ethics approvals to carry out this study were obtained from the Ethical & Research Review Committees of the State Ministry of Health. Ekiti State Teaching Hospital and LAUTECH with the

following numbers: LSMH/ 4148/ VOL1; EKSUTH/ A67/ 2016/01/03; OSHREC/ PRS/ 569T/ 118; LTH/ REC/ 2016/12/08/ 283; MR/GEN. AD/1032/1015. Informed consents of the participants were also sought and obtained.

Consent for publication

All the authors gave consent for the publication of the work under the Creative Commons Attribution-Non-Commercial 4.0 license.

Availability of data and materials

The data and materials associated with this research will be made available by the corresponding author upon reasonable request.

Competing interests

The authors declare that they have no competing interests.

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Authors contributions

OAA conceived, designed, performed data collection, analysis and wrote the manuscript. OES contributed to the data collection, analysis and writing of the manuscript. AAA, EE, OI and AE contributed to the analysis and writing of the manuscript. All authors reviewed the manuscript and approved the submitted final version.

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