Molecular Detection of Mycobacterium ulcerans strains in South-west, Nigeria

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Abstract

Mycobacterium ulcerans infection (Buruli ulcer) is a debilitating disease characterized by extensive and severe destruction of the skin and subcutaneous tissue resulting in the formation of large ulcers. The neglected tropical disease whose mode of transmission is still unknown was regarded as an emerging infectious disease by WHO in 1998. Index cases of BU were first reported in Badan (now in Oyo State), south-west, Nigeria in 1975. The study aimed to identify cases of BU in five states of south-west Nigeria and carry out a phylogenetic analysis of the strains encountered. Here we processed 586 samples from the south-western part of Nigeria for IS4800 of Mycobacterium ulcerans by Nested PCR and RQ-PCR techniques. Randomly selected, non-positve samples were subjected to gene sequencing protocols to identify and determine the strain in the region using the molecular and biochemical tools of modern medicine. The genomes sequenced were deposited in the NCBI Data Bank, assigned accession numbers and then mapped to some reference sequences by BLAST analysis and Phylogenetic analysis was also done. All the sequenced genomes can be designated to belong to Clade A of classical type. Over 1500 nucleotides and high concordance and higher genetic similarity to reference strains from Africa was observed. This corroborates its strong geographical diversity and intra-strains being genetically extremely closely related.

Introduction

Buruli ulcer (BU), which is caused by Mycobacterium ulcerans is a neglected disease of the skin occurring in tropical and subtropical regions of the world. BU has been reported in over 33 countries in tropical and subtropical regions, particularly in Africa, Asia, Australia, South America and the Pacific. It is most prevalent in Central and West Africa while the latter is now regarded as the epicentre of the disease which is regarded as one of the world’s neglected tropical diseases [1]. The mycolactone, a macrolide polyketide that is toxic and immunosuppressive (in nature), produced by the causative pathogen is responsible for the characteristic chronic necrosis of skin and tissue, usually with undermining of the skin ulceration that is closely related to the mycobacteria that cause tuberculosis and leprosy. BU is the third most common mycobacteriosis in immunocompetent humans after tuberculosis and leprosy, and in some communities in Africa, BU has been reported as the presenting site of tuberculosis and become the most common [2]. While the mode of transmission of BU is poorly understood, awareness of the disease among the population and healthcare providers is equally poor thus making diagnosis a challenging exercise. BU usually presents as a papule or papilla or (pre-ulcerative form) on the skin, which, if left untreated, over time, progresses to major ulcerations (ulcerative form) mostly on the body extremities [3]. When BU is untreated, it can lead to permanent disfigurement, functional impairment and disability with attendant stigmatization in the society [3].

Materials and Methods

Being a neglected and obscure disease, community BU awareness/ sensitization sessions were carried out prior to the active community screening in each target states. Swab and Fine Needle Aspiration (FNA) samples were collected from suspected lesions. Nested PCR and RQ-PCR techniques were deployed to confirm BU among the participants. Eight of the samples positive to IS4800 of M. ulcerans were randomly selected and subjected to genomic Sequencing on ABI 3130XL sequencer platform. The chromatograms of the sequences (Fig.1) were viewed using FinchTV analysis software and the matrix base calling was carried out for regions of ambiguities. The nucleotide sequences were thereafter subjected to BLASTn analysis to identify the organism and subsequently submitted to GenBank (accession numbers) after accession Numbers were obtained (Table 1). Two slightly different approaches of phylogenetic analyses were employed.

APPROACH 1 FOR PHYLOGENETIC ANALYSIS: A phylogenetic tree was created using the deposited sequences and reference strains sequences. Fasta versions of all the strains used were obtained from the NCBI database (https://www.ncbi.nlm.nih.gov/). The fasta version of each of the strains were submitted to the FinchTV software package (Fig. 1). The GenBank accession numbers for the nucleotide sequences.

APPROACH 2 FOR PHYLOGENETIC ANALYSIS: Due to high number of target sequences that were all identical to one another within the genome (orthologous), 8 representative sequences from each genome were therefore used. They were labelled as follows: CP0003251 Fragment 1 to Fragment 8

Table 1. GenBank accession numbers for the nucleotide sequences.

<table>
<thead>
<tr>
<th>Fragment</th>
<th>Accession Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>EF164897</td>
</tr>
<tr>
<td>2</td>
<td>AP017635.1</td>
</tr>
<tr>
<td>3</td>
<td>KJ632761</td>
</tr>
<tr>
<td>4</td>
<td>MK359610</td>
</tr>
<tr>
<td>5</td>
<td>MK359609</td>
</tr>
</tbody>
</table>

Table 2. Multilocus Category II BU ulcer in a 43y-oil woman's right lower limb

<table>
<thead>
<tr>
<th>Plate 1</th>
<th>13570 100 80 60 40 20 0</th>
</tr>
</thead>
</table>

Discussion

We screened some clinically suspected Buruli ulcer patients using a nested PCR for the confirmation of the presence of an insertion sequence IS2404 from the genome of Mycobacterium ulcerans, the aetiological agent of Buruli ulcer. Gene: IS2404, which encodes a protein of 327 amino acids, is an insertion sequence from Mycobacterium ulcerans genome [7]. In order to evaluate our strains genetic diversity in comparison with publicly available suitable genome data using comparative genomics, reference genomes of Mycobacterium ulcerans were obtained from the NCBI database (Accession Numbers: L1135168 and CP000325; M. ulcerans subspecies africanae (Accession Numbers: AP017624 and AP017635). We also obtained sequences of M. ulcerans IS4800 genes (Accession Numbers: EF164897, MK359600 and MK359601). The sequences obtained from NCBI were aligned with the 8 sequences from this study and at an approximately 218bp of the IS4800 region of the genome which aligned best (with the sequences from this study was extracted and used to infer a maximum likelihood tree using as the best model according to Bayesian Information Criterion (BIC)).

Conclusions

Both approaches showed the similar results. All the samples were identified as M. ulcerans (through BLAST analysis with NCBI).

References