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Original Research

Identification of genetic relatedness of *Mycobacterium ulcerans* DNA from human and aquatic environmental samples: One Health approach to Buruli ulcer epidemiology

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ABSTRACT

Buruli ulcer (BU) disease is a neglected tropical disease caused by *Mycobacterium ulcerans (M. ulcerans)* pathogen, an environmental mycobacterium, which has affinity to inhabit aquatic niches. This disease causes debilitation mostly in children between 3 to 15 years, preventing them from having smooth schooling. Currently, the integration of One Health approach in tackling health challenges is gaining momentum because of the obvious identification of linkages that would proffer more cost-effective and sustainable solution. In this study, the agent-host-environment molecular inter-relatedness in the transmission of Buruli ulcer disease in selected communities in Ogun State, Nigeria contiguous to Benin Republic; a BU epicentre was explored. Extraction of *Mycobacterium ulcerans* DNA from suspected BU patients, water samples from rivers routinely accessed by the patients and aquatic fauna (fish and crabs) from the same rivers was done. Variable Number of Tandem Repeats (VNTR) was amplified from the extracted DNA using primers targeting loci 6 and 19; genetic markers used in the study of *M. ulcerans* diversity. The findings revealed that locus 19 of the VNTR showed presence of *M.ulcerans* population in water, fish and human samples at the band size of 344bp. Locus 6 further confirmed the initial finding indicating the presence of *M. ulcerans* population at the band size of 510bp in fish, crab, water and human samples. There is an indication of plausible involvement of fish and crab (animal component), water (environmental component) and humans (host component) in the transmission of *M. ulcerans* pathogen. From the findings in this work, a possible transmission pathway of *M. ulcerans* to humans was proposed.

Keywords: Buruli ulcer, molecular epidemiology, *M. ulcerans*, Ogun State.

INTRODUCTION

The exact mode of transmission of Buruli ulcer (BU) disease remains evasive, thus hampering prevention and control strategies in BU endemic regions (Muleta *et al.*, 2021). Global distribution of BU spans across over 33 countries, however, in the West African sub region, incidence of BU is alarming with severe devastating burden especially in children resident in rural communities (Simpson *et al.*, 2019). Prompt reporting and early detection of cases followed by timely treatment of patients is available means of intervention for the poor rural populace who have inadequate or no access to health care (Otuh *et al.*, 2019). Buruli ulcer disease is poverty promoting with attendant socio-economic consequences an off shoot of limited presence of government facilities in high risk zones. Patients often suffer chronic devastating skin ulcers accompanied with bone disfiguration and physical disabilities resulting in rejection and stigmatization (WHO, 2023).

The organism, *Mycobacterium ulcerans*, is a slow-growing environmental bacterium, the aetiologic agent of Buruli ulcer (BU). It is a quickly emerging yet neglected infectious tropical disease that causes a severe skin disease characterized mainly by chronic necrotizing skin ulcers (Kenu *et al.*, 2014; Röltgen & Pluschke, 2020). The lesions often lead to scarring, contracture and in some cases osteomyelitis and potential amputation (Jacobsen & Padgett 2010). Although it is assumed that the majority of BU cases abound in West Africa, the geographic spread is not only limited to the tropical sub-Saharan Africa because cases have been reported from Australia, China, Japan, South America, Mexico and in several islands in the Pacific and Indian oceans, in addition to Papua New Guinea, Malaysia,

Indonesia and Sri Lanka (Dega *et al*., 2000; WHO, 2008; Nakanaga *et al*., 2011; WHO 2016). This disease is presently the third most common mycobacterial human disease, after tuberculosis and leprosy but BU incidence is surpassing that of tuberculosis and leprosy in some West African countries (Narh *et al* 2014; Marion *et al*., 2015). Increased urbanization, agricultural activities, massive land use with attendant distortion of the natural niches of BU causative organism are attributable to increased prevalence of this disease (Huang & Johnson, 2014).

Globally, Buruli ulcer is becoming a disease of immense burden, unfortunately, despite its increasing prevalence around the globe, the epidemiology, mode of transmission, and molecular mechanisms of *M. ulcerans* and the associated disease remain poorly understood (WHO, 2023). Many African countries have reported Buruli ulcer, however recent evidence of endemicity in the West African countries of Ghana, Benin, Cameroon, Côte d'Ivoire, Democratic Republic of Congo (DRC) have been of the rise and is of concern (Phanzu *et al*., 2011; Tabah, *et al*., 2019). In Nigeria, predominant peer-reviewed evidences are clinical reports from hospitals (Chukwukezie *et al*., 2007), however, studies on *M.ulcerans* epidemiology are very few (Ukwajah *et al*., 2016, Otuh *et al*., 2018). In the report by Ukwajah *et* al., 2016, a crude prevalence of 18.7/100,000 was recorded in the pilot study while the study conducted in Ogun State by Otuh *et al*., 2019 revealed active and inactive cases of BU during the survey with a prevalence of 0.01%. There is dearth of information on the national status of BU in Nigeria; however report has it that a considerable number of BU patients visit Benin Republic for treatment pointing out the fact that the BU prevalence might be higher than presumed (Marion *et al*., 2014). This being the case, it is quite important that research directed towards finding the status of BU in different parts of Nigeria should be encouraged. Steps channeled in this direction will lead to several areas in the course of understanding the unexplored areas of research in BU.

Extensive epidemiological research is vital in the efforts to discover the exact mode of transmission and transmission pathways, the geographical spread and prevalence of BU. World Health Organization as part of plans to intensify efforts towards prevention and control of BU encourages broad research across countries (WHO, 2008). However, poor development of BU diagnostic facilities in the endemic West African region especially in Nigeria is a major hurdle and accounts for the dearth of research in BU. Diagnosis of BU includes clinical signs and symptoms, microscopy, culture, histology and molecular methods (Ruf *et al*, 2011; Yeboah-Manu *et al* 2011). These methods present different degrees of sensitivity and specificity. Culture has about 35-50% sensitivity while microscopic staining for acid-fast bacilli and histology give a sensitivity of 40% and 63-90% respectively, however resources for these methods are lacking in most rural remote endemic areas (Phillips *et al*., 2005; Williamson *et al*., 2014). Molecular methods including Variable Number of Tandem Repeats (VNTR) typing method involving Polymerase Chain Reaction (PCR) targeting different VNTR loci (ST1, MIRU1, loci 6 and 19) have been successfully employed to discriminate between strains of *M. ulcerans* derived from environmental samples from African countries (Ghana and Benin) which also revealed strain heterogeneity within the aquatic habitats (Willson *et al*., 2013; Reynaud *et al*., 2015).

Buruli ulcer is associated with proximity to aquatic habitats and isolation of *M. ulcerans* from aquatic environments within endemic areas provides strong clue on possibilities linking to transmission pathways and insight to potential mode of transmission which hitherto have been inexplicable (Williamson *et al*., 2014). However, isolation of *M. ulcerans* from environmental sources is encumbered with other environmental mycobacteria which possesses common characteristic features even at the genetic level as *M. ulcerans* causing false positive results. These mycobacteria equally have in their genome multicopy insertion sequences IS*2404* and or IS*2606* similar with *M. ulcerans* (Fyfe *et al*., 2007; Lavender *et al*., 2008). Another challenging issue with environmental diagnosis of *M. ulcerans* is mycolactone, the major virulence determinant in *M. ulcerans* which is a macrolide that is responsible for the necrosis and immunosuppression characteristic of Buruli ulcer (Stinear *et al*., 2004). This macrolide happens to be produced by some other environmental mycobacteria known as mycolactone-producing mycobacteria (MPM), acquiring mycolactone gene sequence (genes for mycolactone biosynthesis that form a 110kb cluster on a large 174KB plasmid) in their genome same as *M. ulcerans* hence making exact diagnosis difficult (Williamson *et al*., 2014). So, the application of VNTR analysis can be used to identify and type organisms in environmental samples and as well has ability to distinguish between *M. ulcerans* genotypes and *Mycobacteria* genotypes from other MPM such as *M. liflandii* and *M. pseudoshottsii* which are pathogenic to aquatic vertebrates (Williamson *et al*., 2012; Willson *et al*., 2013). The VNTR analysis which is based on analysis of strain differences within a specific geographical area becomes one of the most appropriate molecular tool for the identification of *M. ulcerans* in both human and environmental samples.

The objective of this study is to employ Variable Number of Tandem Repeat (VNTR) molecular genotyping technique to detect *M. ulcerans* DNA in fish, crabs and human samples from the endemic communities in Ogun State Nigeria.

MATERIALS AND METHODS

STUDY LOCATION

The study was conducted in three local government areas (LGA) situated along the northern part of Ogun state; Ipokia, Yewa North, Yewa South LGAs known to have recorded cases of patients with clinical signs and symptoms of BU, which meets the WHO case definition for Buruli ulcer. One river each was selected from each of the LGAs. In Yewa South, Idogo/Ipaja community had Odo-Yewa, Eggua community in Yewa North had Odo-Eggua while in Ipokia LGA, Whekan Topa River was selected. The geographical grid reference of the respective rivers includes, Odo Yewa (06.83566°N and 002.90888°E), Odo Eggua (07.06464°N and 002.89693°E) and Whekan Topa (06.46676°N and 002.75182°E). The three selected rivers were identified as having constant human activities by the people living within the locality. (Fig I)

Ethical approval was sought and obtained from the Ethical Research Committee of Olabisi Onabanjo University Teaching Hospital (OOUTH), Sagamu, Ogun State with the identification number HREC REG. No: NHREC/8/10/2012.

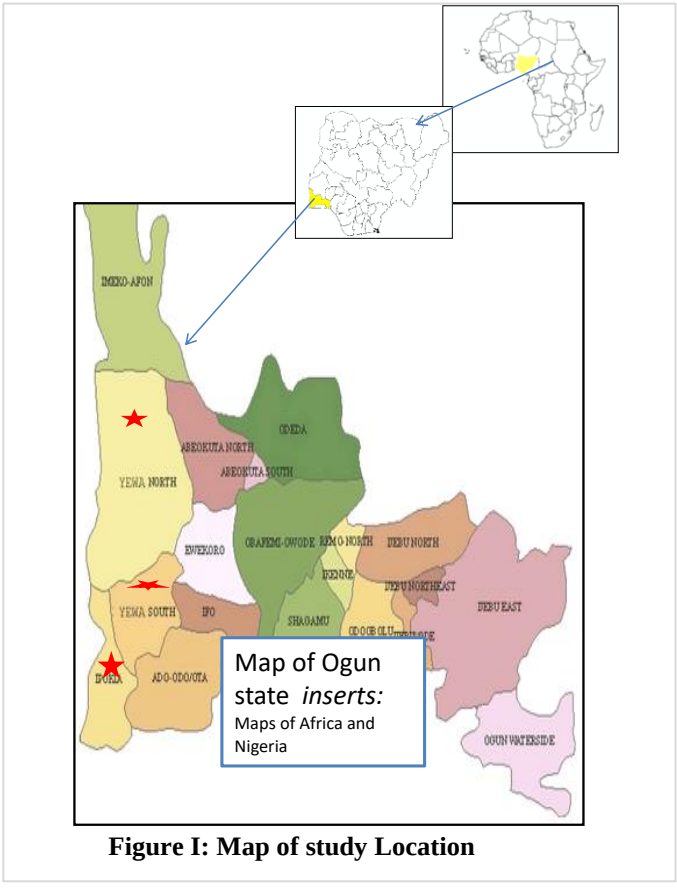


Figure I: Map of study Location

SAMPLE COLLECTION

HUMAN SAMPLE COLLECTION

The human sample collection was carried out in collaboration with the medical personnel from Tuberculosis/Leprosy/Buruli ulcer Unit of the Ogun state Ministry of Health stationed at the Hansen's Disease Centre, Iberekedo Abeokuta, Ogun state. Swab samples from six active BU patients were collected from undermined edges of the ulcers and kept in transport medium. The swab samples were from people living within the three communities of the three LGAs under study. In all, samples from Oke-Odun and Idogo communities in Yewa-South LGA sourcing water from Odo Yewa, Whekan Community (Ipokia LGA) sourcing water from Whekan Topa River and Eggua community in Yewa North LGA with water source from Odo Eggua have one, one, two, two samples respectively.

ENVIRONMENTAL SAMPLE COLLECTION

The environmental samples were collected from the three identified rivers in the study location. From each of the rivers, water and aquatic organisms (fish/crab) were collected. The procedure adopted for environmental sample followed the methods adopted by Williamson *et al*., 2012 and Narh *et al*., 2014 with few modifications.

Water sample collection (collections of water filtrate): This was done from three parts (upstream, midstream and downstream) at each river. At each identified spot in the site about two liters of water was collected by dip sampling from approximately 20cm below the surface water and bacteria concentrated by membrane filteration through a nitrocellulose filter paper of 0.45µm pore size (Whatman Inc). Triplicate collection was done at each of the three parts of each river with designations of A for upstream, B for midstream and C for downstream. At the end three filter papers were obtained from each designate and they were seperately sealed in sterile foil aluminium packets and kept in a cooler for onward transportation to the laboratory. The filter papers were stored at −20°C in the laboratory for further molecular work. At the end nine (9) water samples were preserved.

Fish and crab sample collection: Each river included in this study had fisher men as well as fish sellers/retailers. Fish and crab samples were bought from the local fisher men stored in separate cellophane bags, labeled appropriately and kept in ice. Crabs were obtained only from Whekan Topa River in Ipokia LGA where it was found and in abundance. The other two rivers did not have crabs available. The samples were well labelled and sealed in cellephone, kept in ice in separate coolers and transported to the Wildlife and Fishery Unit laboratory of the Department of Veterinary Public Health and Preventive Medicine, University of Ibadan for dissection and further preservation before molecular studies.

In the laboratory, the fish and crabs were identified and taxonomic classification was carried out before the commencement of dissection. The fish were categorized based on sizes and dissected into skin/scales, gills, flesh and intestines. Likewise the crabs were dissected into shell, gills

and flesh. The different dissected parts from several size categories and according to the sites of collection were each preserved in 95% ethanol. In all, pooled samples of fish and crabs were thirty six (36) in number.

LABORATORY ANALYSIS

HOMOGENISATION

Small portions (25g) of the labeled fish and crab parts were gently and carefully crushed in a sterile ceramic mortar with pestle. Homogenization was done using 1.5ml phosphate buffer (PBS) saline at the pH of 7.4 and stored in 2ml Eppendorf tubes at −20°C.

DNA EXTRACTION

Extraction of DNA from the environmental and human samples was performed adopting the previous procedures by Willson *et al*., 2013 with little modifications. DNA was extracted directly from swabs, homogenized tissue parts from the fish and crabs and filter papers by using the Quick-gDNA MiniPrep™ (D3020, D3021) kit (ZYMO Research Corporation; www.zymoresearch.com). The kit contains genomic lysis buffer, DNA Pre-wash buffer, g-DNA wash buffer, elution buffer, spin columns and collection tubes.

DNA EXTRACTION FROM HUMAN, HOMOGENIZED FISH AND CRAB SAMPLES

DNA was extracted from the human samples using the swab isolation method. This procedure involved the rinsing of the swab stick into a microcentrifuge tube using 500µl of genomic lysis buffer which was vortexed for 4 – 6 seconds and allowed to stand for 5 – 10 minutes. Thereafter the standardized steps as recommended by the manufacture's guideline were strictly followed.

Cell suspension method for DNA extraction was adopted for the fish and crab samples. The homogenized samples in the 2ml Eppendorf tubes were used. The protocol of the DNA kit extractor was strictly followed for the extraction process adopting the steps as above

DNA EXTRACTION FROM WATER SAMPLE (FILTER PAPER)

The stored aluminium foils kept at −20°C were brought out and allowed to thaw. The filter papers were carefully aseptically removed. With the aid of the sterile forceps the filter papers were each transferred into a 1.5ml microcentrifuge tubes. Into the tubes 30µl extraction buffer containing 10mM Tris (pH 8.0), 20mM NaCl and nuclease-free water were added and vortexed briefly and the centrifuged at 10,000xg for a minute. Incubation was done for one hour at room temperature. The resultant extracted fluid was then subjected to DNA extraction using the cell suspension method adopted for DNA extraction of human samples.

THE VNTR PCR FOR HUMAN AND ENVIRONMENTAL SAMPLES

Singleplex molecular typing using two VNTR loci (loci 6 and 19) of the *M. ulcerans* reference genome Agy 99 were chosen for the PCR of DNA extracted from both human and environmental sources according to previous work done by some researchers (Ablordey *et al*., 2005; Williamson *et al*., 2014).

PCR PROTOCOLS FOR THE AMPLIFICATION OF VNTR TYPING IN *M. ULCERANS* (MUVNTR) LOCI 6 AND 19

The first-round PCR used primer pairs: PfkAF (5′-GACCGTCATGTCGTTCGATCCTAGT-3′) as the forward primer and PfkAR (5′-GACATCGAAGAGGTGTGCCGTCT-3′) as the reverse primer for locus 6 amplification while the second-round of PCR used primer pairs: DeaDF (5′-CCGACGGATGAATCTGTAGGT-3′) for the forward while the reverse used DeaDR (5′-TGGCGACGATCGAGTCTC-3′) for the amplification of locus 19. The primers were chosen based on the previous work of Fyfe *et al*., 2007 and Williamson *et al*., 2008. The sequences were designed to flank VNTR loci 6 and 19 primers as were carried out by the above mentioned researchers.

Prior to amplification the master mix was reconstituted following the standardized methods strictly following the manufacturers guideline (Thermo Fisher Scientific™). Final volume of 47µl master mix was eventually used for the amplification with 3 µl of genomic DNA template to make up to 50 µl of the total reaction. Amplification was performed in an Eppendorf gradient thermocycler. There were two cycling phases of one cycle for denaturing phase and thirty five cycles from annealing to the final extension phases. Reaction was left over night at − 4°C in the thermocycler until time for the PCR product analysis.

THE VNTR PCR PRODUCT ANALYSIS USING GEL ELECTROPHORESIS

2% agarose gel was prepared by dissolving 2grammes (2g) of agarose powder in 100ml of TBE buffer (Tris/borate/EDTA buffer). The mixture was heated in a microwave oven at 150°C for 2 minutes. The melted agarose gel was stained using 3µl of ethidium bromide. The melted agarose was cast in a gel mold for polymerization for 30 minutes at room temperature.

Two microliters (2µl) of DNA gel Loading dye (6x) (Thermo Fisher Scientific™) was mixed with 5µl of each VNTR PCR genomic DNA. The resultant mixture was loaded into each well of the agarose gel. The DNA electrophoresis was performed using an electrophoresis machine (Bio RAD™, Country of make) at 100 volts for 60

minutes (Plate 5.2). PCR products sizes were estimated by comparing fragment sizes with a 100-bp DNA ladder (BioLabs) as reference. The gel was first visualized under the UV- transilliminator light (*Clever Scientific World ltd, Country of make*) at wavelength of 365nm and thereafter under a more sensitive Syngene G-Box (model Chemi xx9), where the pictures of gel results were captured.

RESULTS

IDENTIFICATION OF VERTEBRATES AND INVERTEBRATES ENVIRONMENTAL SAMPLES

Taxonomic classification of the vertebrate fish obtained from the three rivers under study showed that from Eggua River (Odo Eggua in Yewa North LGA) two major species of fish were obtained; *Heterobranchus bidorsalis* commonly known as African catfish or eel like fattyfin catfish and *Lamprologus callipterus* belonging to Cichlidae family (Fig II). From Whekan Topa Rriver in Ipokia LGA fish species collected were predominantly long fin catfish (*Chrysichthys auratus*) and few *Sarotherodon (T.) galilaeus* (Mango tilapia) fish species (Fig III). Several invertebrate crabs identified as *Scylla serrata* specie (black crab or giant mud crab) were obtained from only Whekan Topa River. Odo Yewa (Yewa River) in Yewa South LGA had two different fish species *Chrysichthys auratus (long fin catfish) and Oreochromis niloticus* (tilapia).

DETECTION OF *M. ULCERANS* VNTR LOCUS 6 IN HUMAN AND ENVIRONMENTAL SAMPLES

Genomic DNA extracted from human and environmental samples tested positive for MUVNTR locus 6. Some of the environmental samples (Fish, crab and water) from Ipokia and Yewa North LGAs showed bands of different intensities in gel electrophoresis analysis. Band sizes within very close range of 500-510bp (basepair) were identified as positive results. Three positives one for each type of sample from fish obtained from Whekan Topa River tested positive to MUVNTR locus 6. The skin/scale, gill and intestine of medium sized long fin catfish (*Chrysichthys auratus*) were positive. For the samples from crab, only the shell tested positive to MUVNTR locus 6. Water sample from Whekan Topa River was positive for locus 6. Gills and intestinal samples from *Heterobranchus bidorsalis* (fatty catfish) as well as water filtrand obtained from Eggua River had positive MUVNTR locus 6. Of the human samples tested, one each from Whekan and Oke-Odun were MUVNTR locus 6 positive.

DETECTION OF *M.ULCERANS* VNTR LOCUS 19 IN HUMAN AND ENVIRONMENTAL SAMPLES

MUVNTR locus 19 was positive to both human and environmental extracted genomic DNA. Each type of sample (gills and intestine) from same longfin catfish (*Chrysichthys auratus*) from Whekan Topa River tested positive for locus 19. Also samples of fatty catfish (*Heterobranchus bidorsalis*) and *Lamprolugus callipterus* species (intestines and gills) from Eggua River were positive for MUVNTR locus 19. Water samples from Whekan Topa and Eggua Riveres were both positive for locus 19. For the human samples, the same samples that tested positive from Whekan and Eggua for locus 6 were also positive for locus 19. In general, MUVNTR locus 6, percentage positivity for fish, crab, water and human samples were 16.7%, 16.7%, 22.2% and 40% respectively. Conversely, MUVNTR locus 19, percentage positivity for fish, crab, water and human samples were 26.6%, 0%, 22.4% and 40% respectively.

DISCUSSION

This study serves as a preliminary attempt to detect *M. ulcerans* DNA from human and environmental samples in the study areas. An effort which has yielded positive outcome in the detection of MUVNTR loci 6 and 19 previously confirmed in the *M. ulcerans* Agy99 genome (Hilty *et al*., 2007) hence validating the presence of Buruli ulcer disease in Nigeria with the pathogen found in aquatic environments (Gray *et al*., 1969; Oluwasanmi *et al*., 1976; Marsollier *et al*., 2004; Chukwukezie *et* al., 2007; Merritt *et al*., 2010; Williamson *et al*., 2012; Narh *et al*., 2014). Concerted efforts directed towards clinical, ecological, molecular and epidemiological aspects of *M. ulcerans* especially in Buruli ulcer endemic parts of the world has recorded remarkable advancement (Fyfe *et al*., 2010; Ukwajah *et al*., 2016). This study contributes to the ongoing efforts in BU endemic regions by exploring the potentials of BU endemicity in South Western Nigeria using VNTR PCR in confirming *M. ulcerans* presence in the study location. This molecular technique has been confirmed as discriminatory in the typing *M. ulcerans* strains (Williamson *et al*., 2012).

Previous attempt at using molecular technique to detect *M. ulcerans* DNA in Australia and West Africa employed PCR to demonstrate the presence of Insertion sequence (IS) element 2404 present in about 213 copies in the *M. ulcerans* Agy99 genome (Pidot *et al*., 2010). IS2404 PCR performed on DNA extracted from both environmental samples (water, detritus, aquatic insects, plants and small fish) as well as from clinical samples (human) has proved a major progress (Ross *et al*., 1997; Fyfe *et al*., 2007;). However, while IS2404 PCR is highly specific and sensitive for testing diagnostic specimens from humans, its application to the analysis of environmental samples is less straightforward due to PCR inhibitors and the existence of other environmental mycobacteria that may carry IS2404 (Ranger *et al*., 2006). Therefore approaches for increasing confidence in the

Figure II: Fish species from Odo Eggua (Eggua River)

Figure III: Fish species from Whekan Topa River
c: Chrysichthys auratus (long fin catfish), d: Sarotherodon (T.) galilaeus (mango tilapia**)**

Figure IV: Invertebrate crab specie from Whekan Topa River. Scylla serrata (black crab or mud crab)

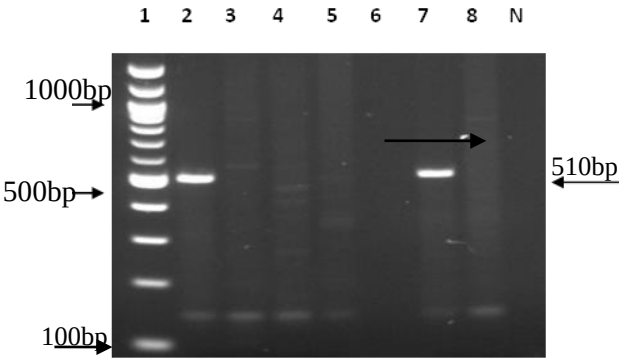
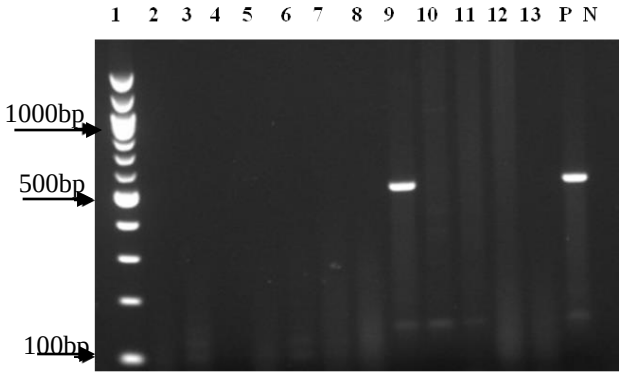
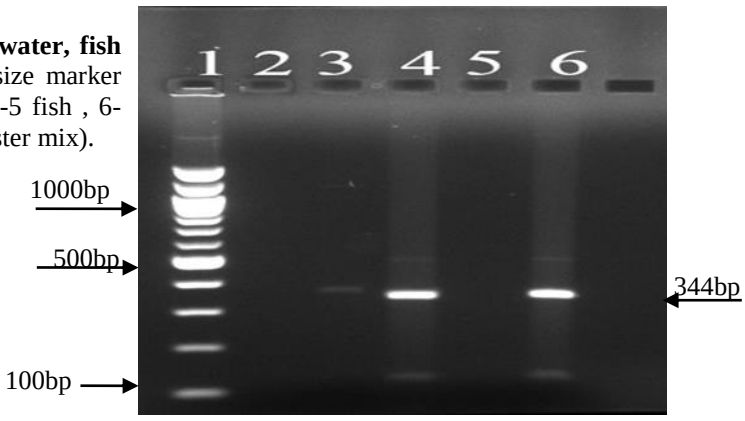


Figure V: MUVNTR locus 6 detection in environmental and human samples from Whekan community (Ipokia LGA). Lanes 1- DNA size marker (100 bp ladder; BioLabs), 2-human (ulcer egdes), 3-water, 4-crab (shell), 5- fish (gill), 6-fish (flesh), 7-fish (intestine), 8- fish (skin), N- negative control (master



FigureVI: MUVNTR locus 6 detection in enviromental and human samples from Oke-Odun (Idogo) community (Yewa South LGA). Lanes 1- DNA size marker (100 bp ladder; BioLabs), 2-8 fish samples, 9-human sample, 10-13 water samples, P- positive control, N- negative control (master mix).

Figure VII: MUVNTR Locus 19 in water, fish and human samples Lanes 1- DNA size marker (100 bp ladder; BioLabs), 2-3 water, 4-5 fish , 6-human sample, 7 - negative control (master mix).



interpretation of PCR- positive tests for environmental samples and for overcoming the issues surrounding primer specificity consists of the use of an internal probe to confirm the identity of a PCR product or the use two or more DNA targets (Fyfe *et al*., 2007). The incorporation of MUVNTR loci 6 and 19 by Williamson and others (Williamson *et al*., 2008) in the study of distribution of *Mycobacterium ulcerans* in BU endemic and non-endemic aquatic sites in Ghana yielded more refined sub-grouping and the finding of a fourth genotype. For this reason VNTR PCR targeting two sensitive and specific loci 6 and 19 in the *M. ulcerans* genome was adopted in this study to ensure high precision in the detection of *M. ulcerans* DNA in the environmental and human samples from the study areas.

Fish from both Whekan and Eggua communities had positive MUVNTR for loci 6 and 19 in their intestinal tissues. As aquatic consumers, fish may concentrate microorganisms such as environmental mycobacteria like *M. ulcerans* thereby participating in the dissemination of the bacilli in the aquatic environment. Marsorllier *et al*., (2004) studied aquatic snails and found out that they possessed concentrated levels of *M. ulcerans* in their tissue. This outcome provides a plausible evidence of likelihood of fish serving as a vehicle (host) in the dissemination and transmission of MU in the aquatic environment. In another environmental study to determine the potential role of fish in the transmission of *M. ulcerans*, Eddyani *et al*., (2004) discovered that fish found positive for *M. ulcerans* DNA all appear to feed on insects or plankton and are believed to concentrate *M. ulcerans* from this food source. This supports the fact that accumulation of *M. ulcerans* in the intestinal tissues of fish is strongly linked to the activities in the trophic levels.

This study equally discovered *M. ulcerans* DNA in water filtrands from surface water collaborating previous work done which also involved detection of *M. ulcerans* not only from surface water but water filtrands from wells, cisterns and ground water (Lavender *et al*., 2008; Vandelannoote *et al*., 2010). In other studies *M. ulcerans* was detected from stagnant water in Australia (Fyfe *et al*., 2007; Johnson *et al*., 2007). With fish and water testing positive there is possibility that they may be reservoirs of *M. ulcerans* hence play a vital role in the transmission of Buruli ulcer in human and animals.

This is the first report of *M. ulcerans* DNA detection from crab (*Scylla serrata*) another aquatic organism probably harboring the pathogen. Several studies have explored other aquatic fauna ranging from amoeba, snails, fish, amphibians to aquatic insects (Eddyani *et al*., 2004; Kotlowski *et al*., 2004; Marsorllier *et al*., 2004; Mosi *et al*., 2008; Amissah *et al*., 2014). Furthermore the detection of MUVNTR locus 6 in crab shells is an indication that crab may also harbor MU pathogen and as such, crabs need to be extensively studied in the epidemiology of BU.

Band sizes of positive MUVNTR locus 19 for water, human and fish are very near (348bp, 340bp and 342bp respectively) likewise that of positive MUVNTR for locus 6 (550bp, 510bp and 500bp respectively). These band sizes are same with *M. ulcerans* strains from Africa and Australia (Lavender *et al*., 2008). There is probability that the strain circulating in the study location (Ogun State) may most likely be of African origin (strain) as the patients from whom the swab samples were collected from obviously never had any contact with Australian environment. The water, fish and human cycle of positivity to the MUVNTR loci may be adduced as a probable evidence of BU transmission pathway. This present work signifies molecular detection of *M. ulcerans* DNA from both human and environment samples conducted in Nigeria which is geared towards finding out if the *M. ulcerans* DNA from humans has genetic relatedness with that from the environment. The positive MUVNTR loci 6 and 19 of humans and environmental samples achieved this aim strongly indicative of genetic diversity of *M. ulcerans* strains circulating in the study areas. Similar to the findings from this study, several reports from West African countries of Ghana, Togo, Benin, Cameroon; Australia, Papua New Guinea and others have recorded success in detecting *M. ulcerans* from environmental sources (Merritt *et al*., 2010; Williamson *et al*., 2012; Garchitorena *et al*., 2015). It is noteworthy that even though Cameroon has no BU reference laboratory, the Country has integrated BU as a notifiable disease in their national program and established nationwide surveillance geared towards ascertaining BU prevalence on a national scale (Tabah *et al*., 2016). Nonetheless Buruli ulcer is not yet a notifiable disease in Nigeria, its awareness level is very low and this perhaps accounts for the less attention towards intensified research and international involvement as compared with BU programme in other West African Sub-region. The Federal government of Nigeria is encouraged to as a matter of urgency pay attention to BU burden in Nigeria especially in the rural areas and sponsor commiserate research on BU epidemiology and control strategies.

CONCLUSION

This study has demonstrated the presence of *M. ulcerans* pathogen in the study area and also proves that the lesions in humans within the study location are not unconnected with the *M. ulcerans* in the environment. Furthermore, maintaining several postulations that *M. ulcerans* is an environmental pathogen with strong affinity to aquatic bodies.

This study elucidates insight into locations, which before now had not been explored, especially in Nigeria, from the viewpoint of Buruli ulcer disease context. Crabs and other aquatic fauna should be explored extensively in molecular

epidemiological studies of BU. VNTR detection of *M. ulcerans* in intestinal tissues of fish is a strong indication of fish serving as a vehicle for the dissemination of Buruli ulcer pathogen in the aquatic environment. There is likelihood of fish serving as a reservoir of *M. ulcerans* pathogen as the gills, skin and intestines all tested positive to MUVNTR loci 6 and 19. Efforts should be intensified in culturing *M. ulcerans* from fish samples as detection of *M. ulcerans* DNA does not confirm the organism's replication in fish. Finally, the validation of the multidisciplinary approach in solving health issues at the environment, animal and human interface has been expounded in this research hence promoting the "One Health One World and One Medicine" agenda.

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CONFLICT OF INTEREST

The author declares no conflict of interest

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