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Index Cases of Buruli Ulcer Disease in Three States of South-West, Nigeria – A Preliminary study

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Abstract

Introduction*: Buruli ulcer (BU) caused by Mycobacterium ulcerans is a severe, necrotizing disease affecting the skin, subcutaneous adipose tissue and occasionally bones. This neglected tropical infection was regarded as an emerging infectious disease by the World Health Organization (WHO) in 1998. BU, as the third most common mycobacterial disease after tuberculosis and leprosy, has been reported in more than 30 countries worldwide. The disease is characterized by the production of mycolactone which is thought to be responsible for the large ulcers associated with this disease. The mode of transmission is poorly understood but the controversial role of aquatic insects and mosquitoes has been proposed. This study, as a part of an ongoing research project, is aimed at determining the genomic epidemiology of Buruli ulcer disease (BUD) in south-west, Nigeria.*

Methodology: *Community-based sensitization awareness and an active case search involving the identification of suspected cases of BU were carried out. Swab and Fine Needle Aspiration (FNA) samples were appropriately collected. Nested PCR protocol was carried out on the samples to confirm the disease.*

Results: *The results showed that a total of 6(60.0%) of the10 samples collected and analyzed were PCR positive toIS2404 of Mycobacterium ulcerans. All the positive cases are adults (6) comprising 4(67%) females and 2(33%) males.*

Conclusion/Recommendation: *The result of this preliminary study is significant as it has, for the first time, documented the presence of BU in three of the six states in south-west Nigeria. The deployment of active case search is an efficient approach that can help in the surveillance of the disease and also reduce the knowledge deficit of BU in our society. The result of this study calls for the need to map out and identify endemic areas to enable the appropriate preventive and control measures to be put in place.*

Keywords*-Buruli ulcer, Mycobacterium ulcerans, case search, preliminary study, south-west Nigeria*

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I. Introduction

Buruli ulcer disease (BUD), a neglected tropical disease caused by the pathogen *Mycobacterium ulcerans*, is an insidious necrotizing disease of the skin, subcutaneous tissue and occasionally bones, occurring in tropical and subtropical countries of the world especially in Central America, Australia, South-East Asia and Africa. [1, 2, 3]BU as an emerging infectious disease of major concern has been reported in more than 30 countries worldwide (Fig 1). and West Africa is now the epicenter of the disease whose mode of transmission is still poorly understood.[4 - 7]. This neglected disease regarded as the third most common mycobacterial disease of humans globally, after tuberculosis and leprosy, primarily affects people in remote and rural areas especially those living around wetlands and swampy areas with stagnant lakes or slow-flowing streams in tropical and subtropical regions of the world. [2, 8]However, BU can also be acquired without wetland exposure.In some affected communities in West and Central African countries like Ghana, Benin, Cote d'Ivoire, Togo, Democratic Republic of Congo (DRC), Congo-Brazzaville and Cameroon, increased incidence rates exceeding that of tuberculosis and leprosy have been reported. [9] The causative bacterium, *Mycobacterium ulcerans* is closely related to the organisms that cause tuberculosis (*M. tuberculosis*) and leprosy (*M. leprae).* Although a co-infection of leprosy and Buruli ulcer does occur but rarely [10] a co-infection with tuberculosis has, till date, not been reported. [11]

There are documented reports of the presence of BUD in Nigeria but there seems to be a dearth of knowledge about BU-endemic areas and the burden of the disease in the country. A questionnaire-based study that was carried out prior to this study indicated a poor level of awareness of Buruli ulcer among the relevant

medical professionals in three states of south-west Nigeria. [12] This shocking discovery coupled with the notable gap in cases between the neighbouring countries and Nigeria and also a recent publication involving Nigerian patients being treated for BU in one of the neighbouring countries (Republic of Benin)[13]prompted us to carry out a comprehensive BU case search in six states of south-west, Nigeria. Buruli ulcer usually presents as painless, or at best, a minimally painful, slowly progressive but brutally disfiguring and crippling disease.In the process of infection, the subcutaneous tissue is destroyed leading, in many cases, to an extensive skin peel-off and subsequent characteristic ulcer with undermined edges.[14] Occasionally, bones are involved and may be destroyed too resulting in osteomyelitis in bones adjacent to a cutaneous lesion [8,15].*M. ulcerans* produces a polyketide toxin called mycolactone, a virulent factor responsible for the pathogenesis of the disease. [16] This is a distinguishing feature of *M. ulcerans* amongst human mycobacteria and has been postulated as a possible diagnostic target but the lipid nature of mycolactone makes it poorly immunogenic.[17].The characterized necrosis of the subcutaneous adipose tissues and widespread debilitation of soft tissues and skin with the development of huge ulcers typically located on body extremities, is as a result of cytotoxic and immunosuppressive activities of mycolactone. [18, 19]Though the mortality rate is low, the morbidity and consequent functional disability of the affected tissue and joints is severe and worrisome. However, BU presents in two major clinical forms; the non-ulcerative lesions (nodules, plaques and oedema) and ulcerated form (ulcers of different sizes).[20] Though it affects all ages, patients of BU in Africa are mostly children under 15 years of age. [13]

Buruli ulcer,particularly if infection is detected early,can be cured.Early case detection and proper management are very important in order to reduce morbidity and thus prevent resultant disabilities and disfigurement. [10]Except in some endemic areas, the diagnosis of BU can be a challenge as diagnosis based on clinical presentations only, can lead to misdiagnosis, thus making confirmatory diagnosis the only option. Currently, the four available methods of diagnosis are PCR for detecting pathogen specific DNA which is usually *IS2404*, microscopy (ZN stain) for detecting acid-fast bacilli, culture to isolate viable organisms, and histopathology but the *IS2404* PCR is considered the gold standard diagnostic tool. However, most of these methods have major drawbacks as they are carried out in Reference laboratories and cannot be deployed in the field where the disease is most prevalent.

The history of Buruli ulcer in Nigeria is quite intriguing just as the fact sheets are gradually emerging. For example, the first cases of BUD in Nigeria were reported in 1967 by Gray et al [21]. Then in 1975, a confirmed case of the disease among a Caucasian family who lived close to a newly constructed dam on the campus of the University of Ibadan in south-west Nigeria was made [22] leading to the identification of 23 more cases within and around Ibadan metropolis. For over two decades thereafter, there was no follow-up search or research on Buruli ulcer in Nigeria until between 1998 and 2000 when the Institute of Tropical Medicine in Belgium confirmed Buruli ulcer cases from samples sent to it from the Leprosy and Tuberculosis Hospital in Moniaya-Ogoja, Cross River State, Nigeria. Then in 2006, a technical team of World Health Organization (WHO) BU experts in conjunction with the national and state health authorities in Nigeria embarked on a 5-day BUD case search in Akwa Ibom, Anambra, Cross Rivers, Ebonyi and Enugu States (South-South and South-East zones of Nigeria) resulting in 14 (38%) confirmed cases of Buruli ulcer out of the 37 suspected specimens harvested from these areas and examined at the Institute of Tropical Medicine, Antwerp, Belgium using the IS2404 PCR protocol.[23] This unexpected outcome was an eye opener indicating a high probability of BU being endemic in some, yet to be identified areas in Nigeria. To further buttress this point, there was an outbreak of Buruli ulcer in Ayamelum Local Government area (a rice growing area) of Anambra State in July, 2010 involving over 300 patients; and there could be more pockets of BU-endemic areas in other parts of the country.

Currently in Nigeria, there is a gross information deficit about Buruli ulcer among both the populace and the healthcare practitioners with the consequent inability to correctly identify Buruli ulcer cases. This bane, coupled with inadequate public health infrastructure, invariably results in the current low surveillance, low monitoring, underreporting and poor infection prevention and control.

This study seeks to identify BU endemic areas in the south-west zone of Nigeria through active case search so as to create more BU awareness, improve the prevention and consequently the control of BU disease in the study areas.

II. Methodology

Study area: This study was carried out in some communities in three states (Lagos, Ekiti and Ondo) in the south-west, Nigeria.

Study population: The study population were people presenting with the clinical manifestations of BU (suspected cases) as identified by WHO standard definitions, in the communities visited.

Study design: The study was a community-based, cross-sectional one to search for BU cases through outreach and sensitization activities by trained personnel. This took the form of gathering the residents of the whole

community at a place within their community on an already agreed date. Such meetings always had the approval of their respective community leaders. The sensitization activities including short talk, distribution of IEC materials and sometimes video shows on BU were carried out followed by the screening for clinically suspected BU cases. The suspects were briefly interviewed before swab specimens were collected from them according to WHO recommendations.Two swab and Fine Needle Aspiration (FNA) specimens each were collected from the edges of ulcerative lesions (below the end of the undermined edges) and centre of non-ulcerative lesions respectively by the trained healthcare workers. The samples were thereafter, taken to the laboratory in the appropriate transport media. Photographs of lesions were taken to generate a BUD album of the study.

III. Results

Both swabs and FNAs samples were collected from 10 suspected Buruli ulcer patients and analyzed, out of which 6(60%) were confirmed PCR positive to IS2404 of *Mycobacterium ulcerans*. Table 1 shows the distribution of the total number of Buruli ulcer patients confirmed positive across all the three states, with Figure 2 expressing them in chart. All the 6(100%) positive cases were adults comprising 4(67%) female and 2(33%) males. 3(50%) were adults below 40 years of age, but older than 30 years while the remaining 3(50%) were above 40 years of age. All the 6 ulcers were located on the lower limbs (Fig 3).

Fig. 4 shows the picture of gel electrophoresis documentations of some of samples at visualization and interpretation stage.

IV. Discussion

Out of the 6 positive cases, 4(67%) were females and 2 (33%) males. This is not in correlation with previous available reports elsewhere in Africa where males predominate [24] and also at variance with a similar study in Japan.[25]In sub-Saharan Africa BU is more common in children <15 years of age [13] but the reverse is the case in this study where all (100%) of the positive cases are adults. However, some studies in Australia and Japan had BUD patients under 15years of age accounting for only 10% and 19% respectively. [25]

All the cases confirmed positive for BUD in this study were already in the ulcerative stage which is in tandem with some statistics obtained in Australia and Japan where less than 10% of cases are diagnosed before ulceration [25, 26]. The inability to recognize the presentations of the disease at the early stage could be responsible for this. All the lesions were on the extremities (the lower limbs) which is in agreement with previous studies [8, 26].

In conclusion, by deploying the nested PCR (IS2404) technique in this preliminary study, index cases of Buruli ulcer disease, though few, were confirmed in three (3) states (Lagos, Ekiti, and Ondo) of south-west Nigeria. As the study continues to cover more states, there is a high probability that more BUD cases would be identified. The results of this pilot study indicate that the incidence of BU disease in the south-west zone may be more prevalent than afore- thought. The present poor knowledge about the disease in the study area may make the determination of the burden of disease difficult; however, adequate awareness and an appropriate diagnosis of the disease are crucial in its prevention and control.

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Ethical Clearance

The Ethical and Research Review Committee of each of the states approved the study. Written informed consent was obtained from all participants in the study and participation was voluntary. Parents of under-aged (minor) participants gave written informed consents on behalf of their wards. Collection of personal and pertinent information from the patients was done at the study sites under the supervision of TBL&BU Supervisors. Patients' names were replaced with numbers to ensure confidentiality.

References

[1]. F. Portaels, W.M. Meyers, A. Ablordey, A.G. Castro, K. Chemlal, P. de Rijk, et al. First cultivation and characterization of *Mycobacterium ulcerans* from the environment. *PLoSNegl Trop Dis, 2,* 2008, e178.

[2]. C.A. Narh, L. Mosi, C. Quaye, S.C. Tay, B. Bonfoh, and D.K. de Souza, Genotyping Tools for *Mycobacterium ulcerans*-Drawbacks and Future Prospects, *Mycobact Dis, 4(2),* 2014, 1000149.

[3]. M.W. Bratschi, M. Ruf, A. Andreoli, J.C. Minyem, S. Kerber, F.G. Wantong, et al, *Mycobacterium ulcerans* Persistence at a Village water Source of Buruli Ulcer Patients, *PLoSNegl Trop Dis, 8(3),* 2014, e2756.

[4]. H.R. Williamson, L. Mosi, R. Donnell, M. Aqqad, R.W. Merritt, and P.L. Small, *Mycobacterium ulcerans* fails to infect through skin abrasions in a guinea pig infection model: implications for transmission, *PLoSNegl Trop Dis, 8(4),* 2014, e2770.
[5]. R.W. Merritt, E.D. Walker, P.L. Small, J.R. Wallace, P.D. Johnson, M.E. Benbow, et al, Ecology and transmission of Buruli ulcer disease: a systematic review, *PLoSNegl Trop Dis, 4 (12),* 2010, e911.
[6]. D.S. Walsh, F. Portaels, and W.M. Meyers, Buruli ulcer: Advances in understanding *Mycobacterium ulcerans* infection, *DermatolClin, 29(1),* 2011, 1-8.
[7]. P. Oluniyi, A.A. Oke, and I.O.O. Komolafe, Presumptive Diagnosis of Buruli ulcer disease based on Clinical Presentations, *J Micro Infec Dis, 7(1),* 2017, 36-41.
[8]. O.O. Komolafe, Buruli ulcer in Malawi – a first report, *Malawi Medical Journal, 13(3),* 2001, 37-39.
[9]. P. Stragier, A. Ablordey, L.M. Bayonne, Y.L. Lugor, I.S. Sindani, P. Suykerbuyk, et al, Heterogeneity among *Mycobacterium ulcerans* isolates from Africa, Emerg Infect Dis,12(5), 2006, 844-847.
[10]. World Health Organization,*WHO Report Weekly. Epidemiology Record,* May 14, 2004.
[11]. K. Asiedu, M. Raviglione, and R. Scherpbier, Buruli ulcer (M*ycobacterium ulcerans* infection) WHO/COS/CPE/GBUI/2000.1 Geneva: WHO (2000).
[12]. F. Ajogbasile, A. A. Oke and I.O.O. Komolafe, Knowledge of Buruli Ulcer Disease among Healthcare Professionals in South-West Nigeria, *Ann Biom Sc* (In Press)
[13]. E.C.K. Marion, A. Adeye, M. Kempf, A. Chauty, and L. Marsollier, Buruli ulcer in South Western Nigeria: A Retrospective Cohort Study of Patients Treated in Benin, *PLoSNegl Trop Dis, 9(1),* 2015, e3443.
[14]. T. Einarsdottir and K. Huygen, Buruli ulcer, *Human Vaccines, 7(11),* 2011, 1198-1203.
[15]. E. Marion, A. Chauty, M. Kempf, et al, Clinical Features of Spontaneous Partial Healing During *Mycobacterium ulcerans* Infection, *Open Forum Infectious Diseases, 3(1),* 2016, ofw013.
[16]. J. En, S. Kitamoto, A. Kawashima, S. Yonezawa, Y. Kishi, N. Ishii, M. Goto, Mycolactone cytotoxicity in Schwann cells could explain nerve damage in Buruli ulcer, *PLoS Neglected Tropical Diseases, 11(8),* 2017, e0005834.
[17]. S.A. Sakyi, S.Y. Aboagye, I.D. Otchere, A.M. Liao, T.G. Caltagirone, D. Yeboah-Manu, RNA Aptamer That Specifically Binds to Mycolactone and Serves as a Diagnostic Tool for Diagnosis of Buruli Ulcer, *PLoSNegl Trop Dis, 10(10),* 2016, e0004950.
[18]. M.T. Silva, F. Portaels, and J. Pedrosa, Pathogenetic mechanisms of the intracellular parasite *Mycobacterium ulcerans* leading to Buruli ulcer, *Lancet Infect Dis, 9(11),* 2009, 699-710.
[19]. D. Walsh, F. Portaels, and W. Meyers, Buruli ulcer (*Mycobacterium ulcerans* infection), *Trans R Soc Trop Med Hyg, 102,* 2008, 969–978.
[20]. C. Capela, G.E. Sopoh, J.G. Houezo, et al, Clinical Epidemiology of Buruli Ulcer from Benin (2005-2013): Effect of Time-Delay to Diagnosis on Clinical Forms and Severe Phenotypes, *PLoS Neglected Tropical Diseases, 9(9),* 2015, e0004005.
[21]. H.H. Gray, and S. Kingma, Mycobacterial skin ulcers in Nigeria, *Trans R Soc Trop Dis Hyg, 61(5),* 1967, 712-714.
[22]. J.O. Oluwasanmi, S. O. Itayemi, and G.O. Alabi, Buruli (mycobacterial*)* ulcers in Caucasians in Nigeria, *Br J PlastSurg, 28(2),* 1975, 111-113.
[23]. O. Chukwuekezie, E. Ampadu, G. Sopoh, A. Dossou, A. Tiendrebeogo, L. Sadiq, L, et al, Buruli ulcer, Nigeria (Letter), *Emerg Infect Dis, 13(5),* 2007, 782-783.
[24]. Q.B. Vincent, M.F. Ardant, A. Adeye, et al, Clinical epidemiology of laboratory-confirmed Buruli ulcer in Benin: a cohort study, *Lancet Glob Health, 2,* 2014, e422-430.
[25]. K. Nakanaga, Y. Hoshino, R.R. Yotsu, M. Makino, and N. Ishii, Nineteen cases of Buruli ulcer diagnosed in Japan from 1980 to 2010, *Journal of Clinical Microbiology, 49,* 2011, 3829-3836.
[26]. WHO, Treatment of *Mycobacterium ulcerans* Disease (Buruli ulcer): *Guidance for health workers*. Geneva 2013; 1-66.

TABLES AND FIGURES

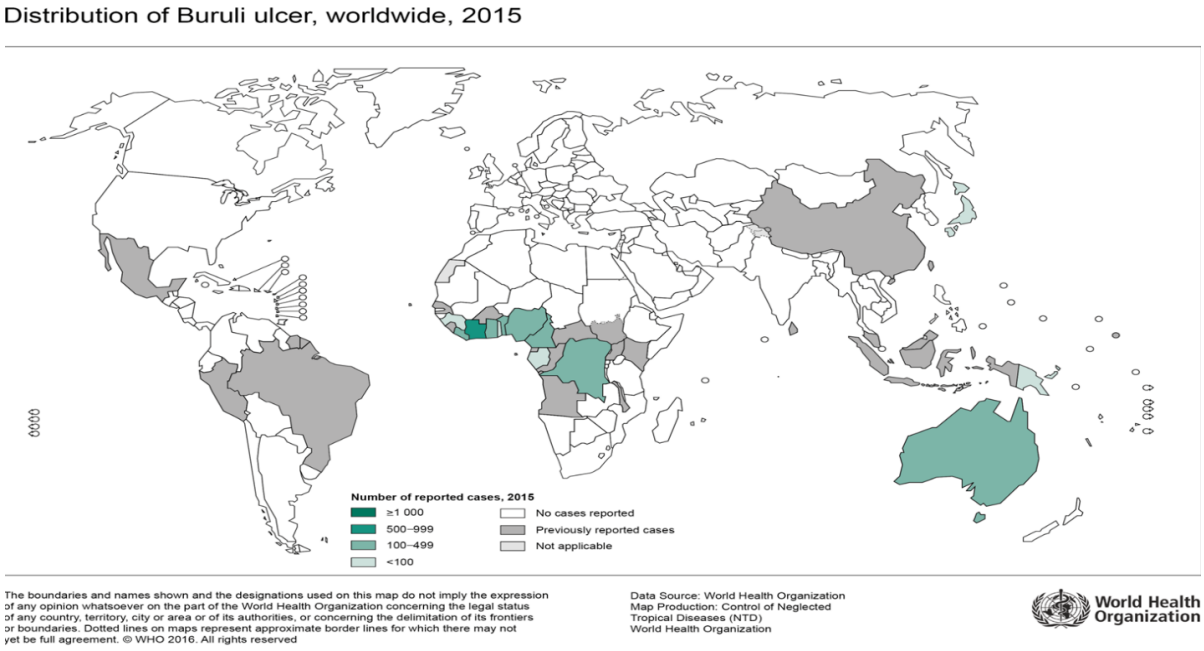

Figure 1: Distribution of Buruli ulcer, worldwide, 2015

Table 1. The distribution of confirmed Buruli ulcer cases by states (n=10)

State	Total Samples Collected	PCR Positive Cases
Ekiti	2	1
Lagos	1	1
Ondo	7	4
Total	**10**	**6 (60%)**

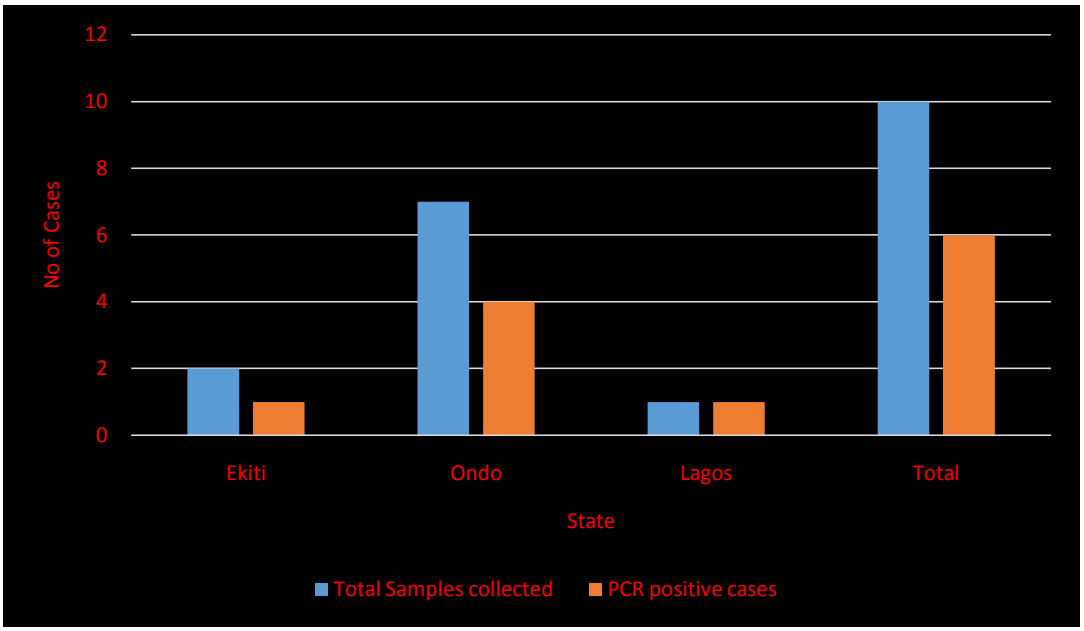

Figure 2. Chart distribution of PCR-positive cases by State

Figure 3: Images of some PCR-confirmed Buruli ulcer cases

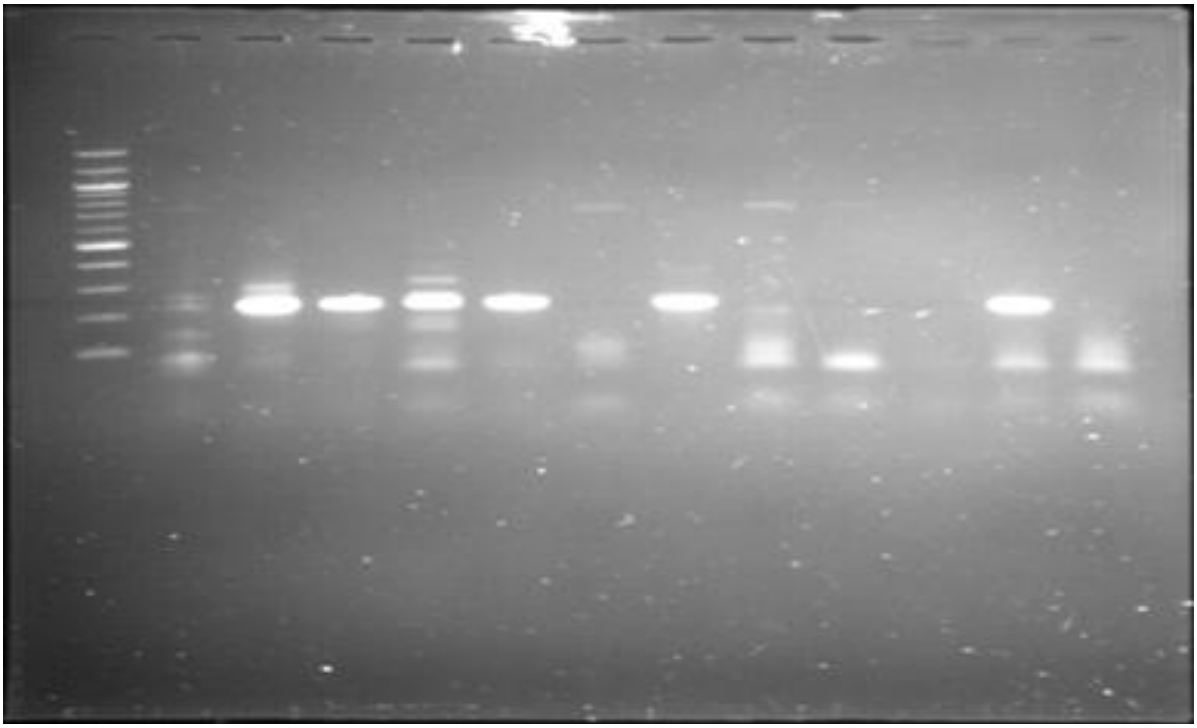

Figure 4. Gel documentation of some of the PCR positive results:
[Lane 1 = 100bp Marker; Lane 2-11 = Samples; Lane 12 = Positive Control; Lane 13= Negative Control].

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