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Prevalence and speciation of malaria parasites among patients attending Ahoada and Amassoma Communities' general hospitals in Rivers and Bayelsa State, Nigeria

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Abstract

Malaria is one of the deadly diseases recognized globally in children and adults especially in Nigeria and some African countries. This study was designed to determine Prevalence and Speciation of malaria parasites from patients attending Ahoada and Amassoma Communities' General Hospitals in Rivers and Bayelsa States respectively. A total of two hundred (200) venous blood collected from Amassoma [101(40 males, 61 Female) and Ahoada [99; 46male, 53 females) into sterile Ethylene Diamine Tetra Acetic Acid (EDTA) bottles from patients attending the above mentioned General Hospitals were immediately transferred to Medical Microbiology Laboratory, Department of Medical Laboratory Science for analysis using Microscopy by staining, Rapid Diagnostic Tests kits (SD Bioline kits; Pf/pan and Pf specific kit) and molecular analysis by Polymerase Chain Reaction (PCR) technique. Twenty (20) positive samples were subjected to Polymerase Chain Reaction Technique for Speciation. Findings showed 141(70.5%) subjects positive to malaria parasites comprised of Children 25(12.5%) between age of 6 and 17 years, adult female 63(31.5%) and male 53(26.5%) between the age of 18 and 90. Also noted were subjects with age ranges of 6-10, 21-25, 51-55 and 86-90 years had the highest percentage prevalence of 100%, followed by 41-45 years with a prevalence rate of 83.3% and the least prevalence was 33.3% from 56-60 years. Observations showed that out of 20 positive samples subjected to molecular diagnosis for Speciation, 17(85%) were *Plasmodium falciparum*, thus revealing their predominance of 90%. In conclusion, *Plasmodium falciparum* was the predominant parasite among Plasmodia species responsible for malaria infection in both Rivers and Bayelsa States. Female and children are vulnerable to malaria infection as revealed in this study. Therefore, the government is encouraged to take women and children's health into consideration by conducting malaria test for them weekly to enable prompt treatment where applicable.

Keywords: Malaria Parasite; *Plasmodium falciparum*; Venous Blood; Polymerase Chain Reaction; Speciation; Rapid Diagnostic Test

1. Introduction

Malaria infection poses a health risk globally especially in developing countries like Nigeria; a disease caused by Plasmodia species belonging to the class of Sporozoa is of public health challenge according to World Health Organization [9]. The etiological agents of malaria infection in humans are *Plasmodium falciparum,P. malariae*, *P. ovale* and *P. vivax*., while *P knowlesi* is zoonotic. Malaria infection is mostly common among the inhabitants of tropical and subtropical countries especially in sub-Saharan Africa [10]. However, *Plasmodium falciparum* is the most common cause of malaria in developing countries especially in Nigeria and is responsible for about 80% to 90% deaths [3; 11]. Children in Africa countries of age less than five (5) years and pregnant women are vulnerable to Malaria infection and 400-900 million are affected annually; this accounts for over 1- 3 million deaths. It has been estimated that the economic burden

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of malaria is extremely high and has affected the countries' economic adversely especially in endemic areas, and the consequent long-term impact is a reduction of Gross National Product (GNP).

P. falciparum is also found in all continents except Europe. Based on the record by World Health Organization (WHO) in World Malaria Report, 2019; 228 million people suffered from malaria in 2018, a slight decrease from 231 million in 2017, 405,000 people died from it. Children under five (5) years of age are most affected and 61% of malaria deaths occurred in this age group. 80% of the infection is found in Sub-Saharan Africa, 7% in the South-East Asia, and 2% in the Eastern Mediterranean. Outside Africa, India has the highest incidence with 4.5% of the global burden, while in Africa Nigeria has the highest incidence of 27%. Presently, Europe is a malaria-free region due to application of insecticide spraying, drug therapy and environmental engineering since early 20th century; this has resulted into total inexistence of these parasites. Early diagnosis is paramount to effective treatment, control and disease surveillance of the malaria infection in endemic regions; this will help in minimizing the morbidity and mortality due to malaria. One of the challenges due to malaria diagnosis is well equipped standard laboratories which could be a hindrance to effective malaria treatment and control. This has led to misuse and abuse of anti-malarial drugs of which the consequence is the development of drug resistance. *P. falciparum is* fatal due to its infectivity on the cerebral region of the body, it needs effective identification and proper differentiation from the other *Plasmodium* species that cause malaria. Microscopy still remains the method of choice in the diagnosis of malaria in endemic areas because it is cost effective. This aspect must be handled by an expert Medical Laboratory Scientist. The sensitivity of microscopy is approximately 10-30 parasites/µL of blood and this is usually a challenge in malaria endemic areas. These factors may result in incorrect speciation and in ability to detect mix infections and low parasitaemia. Since the inception of Rapid Diagnostic Tests (RDTs), there has been a reported improvement in the diagnosis of malaria and several commercially available tests are sensitive, specific, and stable under operational conditions. However, one of the limitations of RDTs is the presence of protein HRP-2 which is based on immune- chromatographic; this only permits rapid diagnosis of *P. falciparum* malaria, hence their clinical usefulness for the diagnosis of other *Plasmodium* spp. (such as P. vivax, P. ovale, and P. malaria) and for monitoring of the therapeutic response is limited. The protein HRP-2 is expressed only by *P. falciparum* and the will negative for others. Many cases of non-*falciparum malaria* may therefore be misdiagnosed as malaria negative. Molecular diagnosis using Polymerase chain reaction (PCR) technique has been reported to be more sensitive and specific and have the ability to detect malaria parasites in patients with low levels of parasitemia with proper identification to the species level. The aim of this study was therefore to compare the specificity and sensitivity of microscopy, the RDTs (SD Bioline kits; Pf/pan and Pf specific kit). And Polymerase Chain Reaction technique (PCR) is used for the prevalence and speciation of *Plasmodium* species in children and adults attending Ahoada and Amassoma General Hospital for treatment in Rivers and Bayelsa States, respectively. The aims and objectives of this study are.

- Collection of Blood samples from Children and Adults attending Amassoma and Ahoada General Hospital for treatment.
- To examine the Blood samples for the presence of malaria parasites using both traditional microscopy method and Rapid Diagnostic Test technique.
- To subject the positive blood samples to Polymerase Chain Reaction in order to determine the Speciation of the malaria parasites.

2. Material and methods

2.1. Study Area

2.1.1. Ahoada and Amassoma in Rivers and Bayelsa respectively

Ahoada local government is located in North West of Port Harcourt, Rivers state in the southern part of Nigeria. It was created in the year 1996 with a land mass of 492km2 and population of about 233,700 citizens as at 2016 census. Their major occupation is trading and farming. The vegetation of Ahoada metropolis is green, with trees and few bushy and swampy areas. Amassoma is one of the towns located in Southern Ijaw Local Government in Bayelsa State, Nigeria with Latitude 4.969882N (4^0C) Longitude 6.109074E (6^0E) and approximately 40km from Yenagoa, the state capital. The town is populated with Ijaw people whose major language and occupation are Ijaw and Fishing respectively. Amassoma has a riverine and estuarine setting, an island called Wilberforce Island with water bodies preventing the development of significant road infrastructure.

2.1.2. Study Design

This study was a cross-sectional descriptive study of the prevalence of malaria parasite in the blood samples collected from patients attending Ahoada and Amassoma community General Hospital in Rivers and Bayelsa State, Nigeria for treatment.

2.1.3. Sample Size

A total of two hundred (200) venous blood samples were collected from patients from Ahoada (99) and Amassoma (101) communities General Hospitals.

2.1.4. Study Population

The study population includes 86 (43%) males and 114 (57%) females.

2.1.5. Materials Used

Glass slide, Microscope, immersion oil, methanol, tap water, cotton wool, applicator stick, Giemsa stain, distilled water, stop watch, staining trough, spreader and staining rack. Malaria Ag "P.f" test strip, EDTA blood sample, Pipette, Buffer solution. The make is SD BIO LINE Malaria Ag P.f (05FK50) and was manufactured by STANDARD DIAGNOSTICS, INC., Republic of Korea. 1.5ml Eppendorf tube, Micro-centrifuge, Vortex, Heating block, NanoDrop1000, Spectrophotometer, desktop, Micropipettes range from (10ul, 10 -100ul7, 100-1000ul), Micropipette tip, Amplified Bio- system Gene-Amp 9700, Bio-Rad (DNA Engine Dyad Peltier Thermal Cycler), Microwave, 1x Tris burette Ethyenediaminetetraacetic acid (TBE), Ultra violet Tran illuminator photo imaging system, Agarose powder, Electrophoretic tank, Electrophoretic machine, Casting try, Combs, Weighing balance, Spatula, Plumb, Conical flask, Ethidium brom, Primer, Master mix, PCR water, Distilled water and normal saline and Polymerase Chain Reaction Machine.

2.1.6. Sample Collection

Approximately 2ml 0f whole blood were collected from symptomatic patients using syringe and dispensed into an EDTA bottles, and standard Giemsa stained thick and thin blood films preparedin the laboratory. Plasmodium infection was determined by RDT and microscopy. Subsequently, the PCR was extracted from the blood.

2.2. Sample Processing

2.2.1. Traditional Microscopy Method Thin and Thick Blood Film Method

Procedure

A drop of fresh blood was gently made to touch one end of a clean grease-free slide. A spreader was used to spread the blood along the glass slide. The spreader was held at a suitable angle of 45oc and pushed along the slide, drawing the blood behind it until the whole of the drop had been smeared, forming a thin film. The thin film was allowed to air dry; then fixed with three drops of of methanol for a few seconds. If the fixation is prolonged, it may be difficult to detect Schiffner's dots and Maurer's cleffs. Forceps was used to place the slide on a staining trough. Three percent (3%) Giemsa solution was prepared in distilled water and added in sufficient quantity to fill the number of staining trough. The stain was poured gently into the staining trough, until all the slides werecompletely covered; this was allowed for 10 minutes out of sunlight, rinsed out with distilled water and air dried at ambient temperature (24.5^0C). The slide was removed from the slide rack with forceps and its back was cleaned and examined microscopically for the presence of plasmodium species (Cheesbrough, 2005). The same procedure was adopted for thick film but was not fixed with ethanol for dehaemoglobinization.

2.2.2. Rapid Diagnostic Test (RDT) Method

Principle

The SD Bioline Malaria Ag *P.f*. test device contains a membrane strip, which is precoated with mouse monoclonal specific to HRP-II of *P. falciparum* on test line "*P.f.*" region. The mouse monoclonal antibodies specific to HRP-II of *P.f*. colloid gold conjugate reacts with the Malaria *Plasmodium falciparum* antigen in the specimen. They move along the membrane chromatographically to the test region "*P.f.*" and form a visible line as the antibody-antigen-antibody gold particle complex with high degree of sensitivity and specificity.

Procedure

- The malaria Ag test device was opened and placed on the bench top as many as possible. With the capillary pipette the blood was drawn into the capillary pipette with capillary action. It was then dropped into blood sample window. Then four drops of the buffer solution were dropped also at its window and allowed to art for 15 minutes for reaction to occur; result was taking.

2.2.3. Polymerase Chain Reaction (PCR)

Plasmodium Species DNA Extraction

Whole Blood DNA Extractions

Procedure

Exactly 150µl of whole blood was pipetted into micro-centrifuge tube. 95µl of 2 x digestion buffer and 5µl of proteinase were added to the micro-centrifuge simultaneously. The content in the tubes were mixed well and then incubated at 55°C for 20 minutes. Seven hundred (700µl) of genomic lysis buffer were added to each tube and thoroughly mixed by vortexing. The mixture were carefully transferred to a Zymo-SpinTM IIC column in a collection tube and centrifuged at 13,900 rpm for 2 minutes. Two hundred (200µl) of DNA pre-wash buffer were added to the spin column in a new collection tube and allowed to centrifuge at 13,900rpm for 2 minutes. Four hundred (400µl) of g-DNA wash buffer was measured and introduced in the spin column. The content was centrifuged at 13,900 rpm for 2 minutes. The spin column was carefully transferred into a clean micro-centrifuge tube. Forty (40µl) of DNA elution buffer was added to the spin column and incubated at 2-5 minutes at room temperature. The final mixture was centrifuged at 8,000 rpm for 2 minutes. The eluted DNA was immediately used for molecular basedapplications or stored at -20°C for future use. The resultant supernatant, containing DNA, was carefully transferred into a pre-labeled 1.5 ml micro-centrifuge tube, excluding chelex, for immediate PCR analysis or stored at -20°C (Nwuzo *et al*, 2015).

2.2.4. Electrophoresis of the PCR Products

The PCR products were separated in 1.5% agarose gels for Touchdown PCR analyses. It was thereafter stained with 1µl Ethidium Bromide (TBE) and allowed to run at 100 mV and 500 mA for 3 hours after which the gel was visualized under a UV light for DNA bands. The PCR amplified fragments of *P. falciparum* genes were 1500bp.

3. Results

Table 1 Demographic/frequency distribution of blood samples collected from Ahoada and Amassoma Communities General Hospitals

	FREQ.	**MALE (%)**	**F/MALE (%)**	**AV. AGE**	**MIN. AGE**	**MAX. AGE**
AHOADA GEN. HOSP	99	46 (46.5)	53 (53.5)	24 YRS	7 MTHS	84 YRS
AMASSOMA GEN. HOSP	101	40 (39.6)	61 (60.4)	27 YRS	1 MTH	89 YRS

Table 2 Demographic table for patients visiting general hospital, Amassoma and Ahoada General Hospitals

(Amassoma)	**FREQ (%)**	**(Ahoada) FREQ (%)**
MARRIED	36 (35.6%)	40 (40.4%)
SINGLE	65 (64.4%)	59 (59.6%)
RDT POSITIVE	41 (40.6%)	41 (41.4%)
RDT NEGATIVE	60 (59.4%)	58 (58.6%)
MICROSCOPY NEGATIVE	36 (35.7%)	46 (46.5%)
MICROSCOPY (+)	19 (18.8%)	10 (10.1%)
MICROSCOPY (++)	5 (4.9%)	5 (5.1%)

MICROSCOPY (+++)	37 (36.7%)	33 (33.2%)
MICROSCOPY (++++)	4 (3.9%)	5 (5.1%)
P.falciparum	65 (64.4%)	53 (53.5%)
P.vivax	Nil	Nil
P.ovale	Nil	Nil
P.malariae	Nil	Nil
Total	10991	99

This Table 2 shows the demographic and level of parasitemia for patients visiting both health Centres Amassoma and Ahoada.

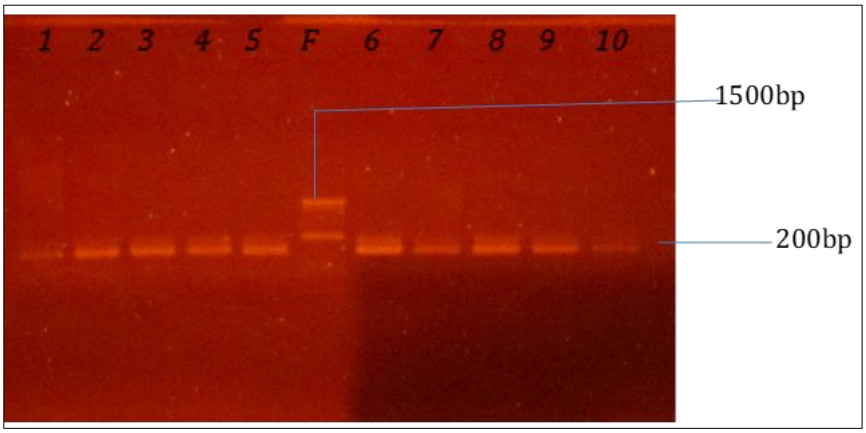

Figure 1 Agarose gel electrophoresis of PFK gene of some selected P. falciparum. Lane 1-10 represents the QnrS gene band (200bp). Lane F represents the 1500bp Molecular ladder of 1500bp

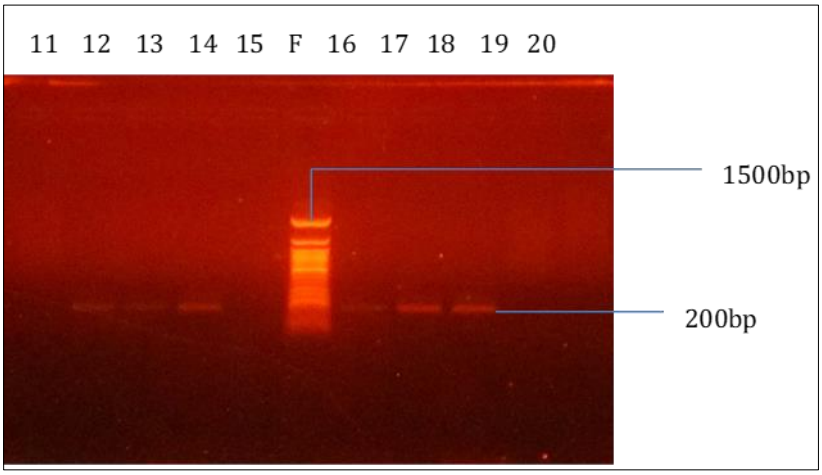

Figure 2 Agarose gel electrophoresis of PFK gene of some selected *P. falciparum*. Lane 12, 13, 14, 16, 17 and 18 represents the PFK gene band (200bp). Lane F represents the 1500bp Molecular ladder of 1500bp

Table 3 Comparative positivity results based on Gender visiting both Hospitals and their Mean age based on Microscopy method

	Frequency	Positivity	Female Positivity	Male Positivity	Mean Age
AHOADA	99	53	31(58.4%)	22(41.5%)	24(24.2%)
AMASSOMA	109	65	33(50.8%)	32(49.2%)	27(26.7%

4. Discussion

Of the 200 (101 Amassoma; 99 Ahoada) Venous Blood samples collected and examined, 82 (41, 50%) from Amassoma and 41(50%) from Ahoada) were positive to Rapid Diagnostic Kit, while 118 (53, 44.9%) from Ahoada and 65(55.1%) from Amassoma) were positive to Microscopy analysis. The age range of the subjects with highest prevalence of 100% among the fifty- three (53) positive samples are 6-10, 21-25, 51-55 and 86-90 years; this is in line with the study reported by Nwuzo *et al*, (2015) of the prevalence of 85.7% in Abakaliki, Southern-Eastern Nigeria. Nwozu *et al,*(2015 also reported that malaria infection is a global health challenge especially in developing countries. Also, the subjects within the age range of 41-45, 16-20, 0-5, 46-50 and 11-15 years had a higher malaria prevalence of 83.3%, 75%, 75% and 69.2% respectivelythan 36-40, 31-35 and 26-30 years which recorded 42,8%, 55.5% and 58.3% respectively; the least prevalence 33.3% was from age range of 56-60 years; this is in concordance with the study reported (50.3%) by Egwunyanga *et al. (*1995); 44.6% by Kolawole *et al*. (2009) both in Jos and 58.6% by Ekanne *et al*, (2008) in Calabar, University Teaching Hospital in children. Observation indicates that females had a high percentage prevalence of (52.3%), this is in agreement with the work done by Jimoh *et al*, (2004) which reported a prevalence of 49.1% in Illorin, Kwara State and Mackay(2008) reported 54.3%.

Based on the molecular analysis carried out on the positive blood samples for Plasmodia speciation using Polymerase Chain Reaction technique, it was observed that *Plasmodium falciparum* was the most prevalent species responsible for malaria infection in the study areas Amassoma and Ahoada in Bayelsa and Rivers state respectively. Greenwood *et al*, (2005) stated that malaria affects 400-900 million people annually and is responsible for over 1-3 million mortality, especially among African children of less than five years of age, likewise adults (especially pregnant women) are vulnerable to malaria infection and can easily transmit same due to their asymptomatic expression in endemic areas. Gething (2010) stated further that*Plasmodium falciparum* is the most important causes of malaria infection which results into about 80% to 90% mortality in Africa especially Nigeria. As of the World Health Organization World Malaria Report 2019, there were 228 million cases of malaria worldwide in 2018, resulting in an estimated 405,000 deaths.

5. Conclusion

In conclusion, these findings have revealed the females having higher percentage prevalence of malaria infections than their male counterparts. Also the high malaria infection was discovered among the subjects of 6-50 years, with minimum age of 6 to maximum of 50 having 100% prevalence; the least was among the subjects of age range 31-90 years. It was shown that some RDT kits could only pick histidine – riched protein which shows that it is only malaria parasites with histidine – riched protein that the kit would record as being positive; this due to the number of positivity of RDT (82) when compared with microscopy (118). Ahoada had a higher malaria infection than Amassoma communities patients examined. *Plasmodium falciparum* was the dominant parasite among the Plasmodia species responsible for malaria infection in these two study States (Bayelsa and Rivers). Therefore, this work has x-rayed the prevalnce of malaria infection in these two study communities among the inhabitants.

Recommendation

Effective awareness should be created to urban residents on mosquito breeding site for identification and removal from time to time. Furthermore, they should ensure the provision of additional mosquito bed nets, fumigation indoor residual spray, windows and doors protected with mosquito treated net and enforcement of law on frequent public sanitation. Pregnant women should be well treated when infected with malaria parasites to avoid the parasites crossing the placenta to the foetus. Blood for transfusion should be well screened before transfusion. Travelers should be encouraged to use chemoprophylaxis before and during travelling to malaria-endemic area; this is important in the management and control of malaria in Nigeria.

Compliance with ethical standards

Acknowledgments

We appreciate the staff and the management of Ahoada and Amassoma Community Hospitals Rivers and Bayelsa states respectively for the opportunity given to us to collect the venous blood samples used in this study. Also we acknowledge the staff of department of medical laboratory science, medical microbiology option, Niger Delta University for allowing us to use their Molecular Laboratory for the analysis.

Disclosure of conflict of interest

No conflict of interest.

Funding

Self-funding.

Authors' contributions

Olorode O. A and Olulu F. F. designed and conducted the experiment; Ogba O.M analyzed the data and vetted the overall results

Statement of ethical approval

Ethical approval was obtained from Hospital Management Board Port Harcourt, Rivers State, Nigeria. An easy-to-read and friendly questionnaire was provided for the collection of demographic and clinical data. A physical examination with a clinical note of any reported sign or symptom was done by a clinician.

Statement of informed consent

Informed consent was obtained from all individual participants included in the study.

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