# EPIDEMIOLOGY, PREVALENCE AND DIAGNOSIS OF FALCIPARUM MALARIA WITH THE MOLECULAR SURVEILLANCE FOR ANTIMALARIAL DRUG RESISTANT GENE MARKERS IN AKURE, NIGERIA

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# EPIDEMIOLOGY, PREVALENCE AND DIAGNOSIS OF FALCIPARUM MALARIA WITH THE MOLECULAR SURVEILLANCE FOR ANTIMALARIAL DRUG RESISTANT GENE MARKERS IN AKURE, NIGERIA

**by**

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## LIST OF SYMBOLS AND ABBREVIATIONS

| | |
|---|---|
| % | Percentage |
| ° | Degree |
| ACT | Artemisinin-based Combination Therapy |
| AL | Artemether -Lumefantrine |
| AOR | Adjusted odd ratio |
| AQ | Amodiaquine |
| AS-AQ | Artesunate-Amodiaquine |
| bp | Base pair |
| CDC | Centre for Disease Control |
| CM | Case management |
| CQ | Chloroquine |
| DBS | Dried Blood Spot |
| DHFR | Dihydrofolate Reductase |
| DNA | Deoxyribonucleic acid |
| DVS | Dominant Vector species |
| EDTA | Ethylenediaminetetraacetic acid |
| EFA | Immunofluorescence Assay |
| ELISA | Enzyme-linked immunosorbent Assay |
| FMOH | Federal Ministry of Health |
| FUTA | Federal University of Technology, Akure |
| g | Gram |
| GST | Glutathione-S-Transferase |
| HDA | Helicase-dependent amplification |

| | |
|---|---|
| HF | Halofantrine |
| HIV | Human Immunodeficiency Virus |
| ICZN | International Commission on Zoological Nomenclature |
| IgG | Immunoglobulins G |
| IgM | Immunoglobulins M |
| IMM | Integrated Malaria Management |
| IPT | Intermittent Preventive Treatment |
| IRS | Indoor Residual Spray |
| ITN | Insecticide Treated Nets |
| IVC | Integrated Vector Control |
| K | Cohen's Kappa |
| LAMP | Loop-mediated Isothermal Amplification |
| LED | Light Emitting Diode |
| LLINs | Long-Lasting Insecticide Nets |
| LUM | Lumefantrine |
| MQ | Mefloquine |
| MRDDS | Malaria Rapid Diagnostic Devices (MRDDS) |
| NASBA | Nucleic acid sequences based amplification |
| NATs | Nucleic Acid Amplification Technique |
| NGN | Nigerian Naira |
| NMCP | National Malaria Control Program |
| NMEP | National Malaria Elimination Programme |
| NPC | Nigeria Population Commission |
| nPCR | nested Polymerase Chain Reaction |
| NPV | Negative predictive value |

| | |
|---|---|
| PCV | Packed Cell Volume |
| *pfcrt* | *Plasmodium falciparum chloroquine resistance transporter* |
| *PfEMP1* | *Plasmodium falciparum Erythrocyte Membrane Protein 1* |
| *pfhrp2* | *Plasmodium falciparum histidine-rich protein 2* |
| *pfk13* | *Plasmodium falciparum kelch 13* |
| *pfmdr* | *Plasmodium falciparum multidrug resistance 1 gene* |
| pH | power of Hydrogen (potential of Hydrogen) |
| PLDH | *Plasmodium* Lactate dehydrogenase |
| PMA | *Plasmodium* Aldolase |
| PMV | Patent Medicine Vendors |
| PP | personal Protection |
| PPV | positive predictive value |
| PQ | Piperaquine |
| QN | Quinine |
| RBC | Red Blood Cell |
| RDT | Rapid Diagnostic test |
| RFLP | Restriction Fragment Length Polymorphism |
| SDA | Strand displacement amplification |
| SMC | Seasonal Malaria Chemoprevention |
| SNP | Single Nucleotide Polymorphism |
| SP | Sulfadoxin-pyrimethamine |
| sp. | species |
| SPSS | Statistical Package for Social Sciences |
| SSU-rRNA | Small sub-unit ribosomal ribonucleic Acid |
| TES | Therapeutic efficacy study |

| | |
|---|---|
| TTM | Transfusion transmitted Malaria |
| WGA | Whole genome amplification |
| WHO | World Health Organization |
| μL | Microliter |

## LIST OF APPENDICES

**EPIDEMIOLOGI, PREVALENS DAN DIAGNOSIS MALARIA FALCIPARUM SERTA PENGAWASAN MOLEKUL UNTUK PENANDA GEN RINTANG UBAT ANTIMALARIA DI AKURE, NIGERIA**

## ABSTRAK

Malaria ialah penyakit parasit yang dahsyat bagi masalah kesihatan awam utama di seluruh dunia, terutamanya di negara sub-Sahara Afrika di mana Nigeria menyumbang kepada beban tertinggi penyakit malaria. Pada masa ini, maklumat tentang epidemiologi, diagnosis dan prevalens penanda gen rintangan ubat antimalaria yang mana pengurusan dan kawalan malaria boleh berasaskan adalah terhad, terutamanya di Akure, Nigeria. Oleh itu, kajian keratan rentas rawak dan berasaskan hospital telah dijalankan untuk menyiasat epidemiologi dan diagnosis malaria falciparum dan pengawasan molekul untuk penanda gen rintangan ubat antimalaria di Akure, Nigeria. Sebanyak 601 sampel darah telah dikumpulkan daripada peserta sukarela dan diperiksa melalui teknik pemeriksaan mikroskopi piawaian parasitologi, rapid diagnostik test (RDT), tindak balas rantai bersarang-polimerase (PCR bersarang), dan sekatan serpihan panjang polimorfisme (RFLP). Maklumat peserta seperti pembolehubah demografi, sosioekonomi dan persekitaran telah dikumpul melalui soal selidik yang telah disusun dengan baik dan telah diuji terlebih dahulu. Kajian ini mendedahkan bahawa hanya *P. falciparum* yang lazim di kawasan kajian dan prevalens keseluruhan secara pemeriksaan mikroskopi, RDT dan PCR bersarang masing-masing adalah 64.89%, 65.7% dan 67.39%. Sementara itu, jumlah purata ketumpatan parasit geometri sebanyak 1096.93 parasit/μL telah direkodkan. Kumpulan umur ≤12 tahun mempunyai kelaziman malaria tertinggi iaitu 77.0% dan purata kepadatan parasit geometri sebanyak 1891.21 parasit/μL darah. Ia juga didapati

bahawa kelaziman malaria berbeza dengan ketara mengikut jantina, dengan lelaki mempunyai kelaziman malaria yang lebih tinggi dan kepadatan parasit sebanyak 69.5% dan 1221.11 parasit/μL darah (*P<0.05*) berbanding perempuan. Analisis regresi logistik multivariat menunjukkan bahawa malaria secara signifikan dikaitkan dengan kumpulan umur ≤12 tahun, lelaki, bertani sebagai pekerjaan, memperoleh pendapatan ≤ NGN 30,000 (US$ 75) sebulan, mempunyai genotip AA, menggunakan sungai sebagai sumber air utama seisi rumah dan mempunyai tumbuh-tumbuhan di sekitar rumah manakala faktor perlindungan utama termasuklah tidur di bawah kelambu dan menggunakan air paip sebagai sumber air utama untuk seisi rumah. Tambahan pula, keberkesanan diagnostik mikroskopi dan *Plasmodium falciparum* histidine kaya protein 2 (*pfhrp2*) RDT adalah tinggi dengan ketara. Anggaran kepekaan, kekhususan, Nilai Ramalan Positif (PPV), Nilai Ramalan Negatif (NPV), ketepatan, indeks j Youden, Kappa Cohen (K) mikroskopi dan RDT masing-masing ialah 96.30, 100.00, 100.00, 92.80, 90.96, 97.96, 97.96. dan 95.06, 94.90, 97.47, 90.29, 95.01, 0.8996, 0.88. Tambahan lagi, daripada 250 sampel yang diperhatikan untuk kelaziman gen rintangan ubat, hampir semua sampel (99.2%) membawa alel jenis liar gen *pfk13* dengan hanya dua pencilan (0.8%) yang membawa satu alel mutasi C469C yang sinonim. Begitu juga, alel jenis liar (76K) gen *pfcrt* mempunyai prevalens yang lebih tinggi sebanyak 60.4% berbanding alel mutasi (76T) dengan 39.6%. Selain itu, kelaziman alel *pfmdr1* 86Y mutasi ialah 14.0% manakala *pfmdr1* 184F mutasi ialah 17.2%. Kesimpulannya, penyakit malaria kekal sebagai masalah kesihatan awam utama di kawasan kajian dan oleh itu strategi Pengurusan Malaria Bersepadu (IMM) bersesuaian yang menggabungkan penggunaan kelambu, semburan sisa dalaman, pengurusan sumber larva, dan kemoterapi harus digunakan. Akhir sekali, terdapat keperluan untuk pengawasan berterusan untuk memantau kemunculan dan penyebaran malaria falciparum yang rintangan kepada ubat.

# EPIDEMIOLOGY, PREVALENCE AND DIAGNOSIS OF FALCIPARUM MALARIA WITH THE MOLECULAR SURVEILLANCE FOR ANTIMALARIAL DRUG RESISTANT GENE MARKERS IN AKURE, NIGERIA

## ABSTRACT

Malaria is a devastating parasitic disease of major public health problem worldwide, particularly in sub-Saharan African countries where Nigeria accounts for the highest burden of malaria disease. Currently, information on the epidemiology, diagnosis and prevalence of antimalarial drug resistant gene markers upon which malaria management and control could be based is scarce, particularly in Akure, Nigeria. Therefore, a randomized cross-sectional and hospital-based study was conducted to investigate the epidemiology and diagnosis of falciparum malaria and the molecular surveillance for antimalarial drug resistant gene markers in Akure, Nigeria. A total of 601 blood samples were collected from volunteered participants and examined through standard parasitological techniques of microscopy examination, rapid diagnostic test (RDT), nested polymerase chain reaction (nested PCR), and restriction fragment length polymorphism (RFLP). The participants information such as demographic, socioeconomic and environmental variables were collected through a structured and pre-tested questionnaire. This study revealed that only *P. falciparum* was prevalent in the study area and the overall prevalence by microscopy, RDT and nested PCR were 64.89%, 65.7% and 67.39%, respectively. Meanwhile, the total geometric mean parasite density of 1096.93 parasite/μL was recorded. The age group ≤12 years had the highest malaria prevalence of 77.0% and geometric mean parasite

density of 1891.21 parasite/μL of blood. It was also found that malaria prevalence differed significantly by sex, with males having higher malaria prevalence and parasite density of 69.5% and 1221.11 parasite/μL of blood (*P<0.05*) as compared to females. The multivariate logistic regression analysis showed that malaria is significantly associated with the age group ≤12 year, male, engaging in farming as an occupation, earning income of ≤ NGN 30,000 per month (US$ 75), having genotype AA, using rivers as the major household water source and having vegetation around homes while the major protective factors include sleeping under mosquito bed net and using tap water as the major household water source. Furthermore, the diagnostic efficacy of microscopy and *Plasmodium falciparum* histidine-rich protein 2 (*pfhrp2*) RDT was significantly high. The estimates of sensitivity, specificity, Positive Predictive Value (PPV), Negative Predictive Value (NPV), accuracy, Youden's j index, Cohen's Kappa (K) of microscopy and RDT were 96.30, 100.00, 100.00, 92.89, 97.50, 0.963, 0.94 and 95.06, 94.90, 97.47, 90.29, 95.01, 0.8996, 0.88, respectively. Moreover, out of the 250 samples observed for the prevalence of drug resistance genes, nearly all the samples (99.2%) carried the wild type allele of the *pfk13* gene with only two isolates (0.8%) carrying one synonymous C469C mutant allele. Similarly, the wild type allele (76K) of the *pfcrt* gene had higher prevalence of 60.4% compared to the mutant allele (76T) with 39.6%. Additionally, the prevalence of the mutant *pfmdr1* 86Y allele was 14.0% whereas the mutant *pfmdr1* 184F was 17.2%. In conclusion, malaria disease remains a major public health problem in the study area and as such appropriate Integrated Malaria Management (IMM) strategy which combines the use of mosquito net, indoor residual spray, larval source management, and chemotherapy should be employed. Finally, there is a need for constant surveillance to monitor the emergence and spread of drug-resistant falciparum malaria.

# CHAPTER 1

# INTRODUCTION

## 1.1 Research background

Malaria is a disease of major public health problem caused by the protozoan parasite of the genus *Plasmodium* (WHO, 2015). It is one of the leading devastating parasitic diseases worldwide, especially in the sub-Sahara African countries. Malaria causes high morbidity and mortality rate in many people, particularly in children and pregnant women (WHO, 2021). It is estimated that about 3.2 billion people are at risk of contracting malaria infection worldwide (WHO, 2015). In 2020, the number of malaria cases was approximately 241 million with 627,000 deaths recorded globally out of which Africa alone accounted for approximately 95% (228 million) of all malaria cases and 96% (602,000) of all malaria deaths (WHO, 2021). Meanwhile, the percentage of total malaria deaths in children below 5 years of age was 77% (482,790 deaths). Thus, a child dies from malaria disease every minute of every day while an adult dies from malaria every three minutes of every day (WHO, 2021).

Malaria is transmitted throughout Nigeria with 97% of the people exposed to malaria disease. This has resulted in an estimated financial loss of about NGN132 billion (US$660 million) cost of treatment, prevention and loss of man-hours annually (FMOH, 2007; Okonko *et al*., 2009, 2010). Moreover, an estimated 55% of all malaria cases were recorded in only six countries which include Nigeria (27%), the Democratic Republic of the Congo (12%), Uganda (5%), Mozambique (4%), Angola (3.4%), and Burkina Faso (3.4%) thus bearing the greatest burden of malaria disease (WHO, 2021). Similarly, the same group of six countries accounted for over half of all malaria deaths and these include Nigeria (27%), the Democratic Republic of the Congo (12%),

Uganda (5%), Mozambique (4%), Angola (3%), and Burkina Faso (3%). Nigeria alone account for about a quarter (27%) of global malaria cases and death, thus bearing the greatest burden of malaria disease (NMCP, 2010; WHO, 2014; WHO, 2021). In 2009, it was estimated that 66% of all hospital visits and at least 30% of hospitalizations are due to malaria (NPC, 2009). In the Southern part of Nigeria, transmission occurs all year-round while in the Northern part of Nigeria, it is more seasonal (Nanvyat *et al*., 2017). Almost all malaria cases in Nigeria are caused by *Plasmodium falciparum* which is the leading cause of death in Africa.

The four major species of *Plasmodium* which have been recognized to infect humans include *Plasmodium falciparum, Plasmodium vivax, Plasmodium malariae* and *Plasmodium ovale* among which the most prevalent, virulent, and deadly one is *P. falciparum* (Duchemin *et al*., 2001; Caraballo, 2014; WHO, 2017). In addition to the four major species, it has become evident that *Plasmodium knowlesi,* a parasite that typically infects forest macaque monkeys, can be transmitted by the *Anopheles* mosquito to cause malaria in humans in Southeast Asia, particularly in Malaysia. *Plasmodium knowlesi* infections are frequently misdiagnosed microscopically as *P. malariae*.

The malaria parasite is transmitted through the bites of *Plasmodium*-infected female *Anopheles* mosquitoes during a blood meal. Out of 430 *Anopheles* species, only 41 are dominant vector species/species complexes (DVS), capable of transmitting malaria at a level of major public health concern worldwide (Hay *et al*., 2010; Sinka *et al*., 2012). The most common species in Africa are *Anopheles gambiae, An. arabiensis,* and *An. funestus* (Besansky *et al*., 2004).

The control and elimination of malaria disease in endemic settings are heavily dependent on accurate and prompt diagnosis. Meanwhile, malaria diagnosis by microscopy and Rapid Diagnostic Tests (RDTs) have been recommended for detecting all suspected malaria cases in Nigeria by the Nigerian malaria control policy (FMOH, 2005). The microscopy technique is capable of quantifying parasite density and detecting various parasite species, albeit it is highly laborious and requires expert microscopists and such experts are unavailable or few in most of the health care centres (Abdulkadir *et al*., 2015). In such settings, an RDT can serve as a rapid and early malaria detection tool (Wongsrichanalai *et al*., 2007). Hence, RDT was recommended and introduced for malaria diagnosis in Nigeria. In view of this, the national malaria control strategic plan has set the goal of reducing malaria-related mortality in Nigeria by 50% with 80% malaria diagnosis of all patients ≥ 5 years through the RDTs (Abdulkadir *et al*., 2015). However, monitoring the efficacy of RDT of choice is highly important for accurate diagnosis. The most commonly employed RDT is that which is based on the detection of histidine-rich protein 2 antigen, specifically for *P. falciparum* (Uyoga *et al*., 2021). Rapid diagnostic test (RDT) is advantageous because it is simple, rapid and requires little expertise to perform (Berzosa *et al*., 2018).

Despite the devastating effect of malaria on public health globally, there is no commercially available malaria vaccine. Therefore, the current malaria control and management strategy is basically dependent on the use of antimalarial drugs. However, the potency and success of available, cheap, and safe antimalarial drugs are being threatened and undermined by the emergence of *P. falciparum* drug resistance. Chloroquine-resistant *Plasmodium* parasites are present in most areas where malaria is endemic including Nigeria (Breman *et al*., 2004). Consequently, the decline in the efficacy of chloroquine has led to the use of alternative antimalarials, such as

antifolates (an example is sulfadoxine-pyrimethamine (SP) or Fansidar™), amodiaquine, and mefloquine which also became ineffective due to parasite drug resistance. Therefore, Artemisin-based Combination Therapy [an example is artemether-lumefantrine (AL) and artesunate-amodiaquine (AS+AQ)] was later recommended and adopted as the first and second-line treatment for acute uncomplicated malaria in endemic countries (Nosten and White, 2007). Furthermore, the use of combination therapy instead of monotherapy was recommended by WHO for the management and control of falciparum malaria in order to prevent the development of falciparum-resistant strains. It is apparent that the ACTs have proven very effective over the years as they saved many lives from malaria disease and death (Enserink, 2007).

Despite the effectiveness of ACTs, studies have however shown the emergence and development of delayed clearance phenotypes, also known as artemisinin resistance in *P. falciparum*. This artemisinin resistance which is marked by mutations in the propeller domain of *P. falciparum kelch 13* (*Pfkelch13*) protein has spread across the Greater Mekong Subregion (GMS) of Southeast Asia such as western Cambodia, Myanmar, Thailand and Vietnam (Noedl *et al*., 2008; Dondorp *et al*., 2009; Ashley *et al*., 2014). Similarly, artemisinin resistance has been reported in other parts of the world such as Guyana, Papua New Guinea, and India leading to serious clinical outcomes (Chenet *et al*., 2016; Das *et al*., 2019; Miotto *et al*., 2020). Though artemisinin resistance is uncommon in Africa, reports of recently conducted studies have shown indigenous or local emergence of artemisinin resistance in Rwanda (Uwimana *et al*., 2020, 2021) and northern Uganda (Asua *et al*., 2020; Balikagala *et al*., 2021). The resistance developed by *P. falciparum* to ACTs has become a serious public health problem in malaria-endemic regions, particularly in Africa where it could

lead to a highly devastating impact. Thus, to control and manage the emergence and development of drug resistance in *P. falciparum*, several molecular markers of antimalarial drug resistance have been identified and developed. These include *Plasmodium falciparum chloroquine resistance transporter*, (*pfcrt)* and *Plasmodium falciparum multidrug resistance gene 1*, (*pfmdr1)* for chloroquine, amodiaquine and lumefantrine (Foote *et al*., 1990; Sisowath *et al*., 2007), and mutation in the *Plasmodium falciparum kelch 13* (K13)-propeller domains (*pfkelch13* mutation) for resistance against artemisinin (Ariey *et al.,* 2013).

The susceptibility and resistance of malaria parasites can be detected by some methods which include the use of *in vivo* assays, *in vitro* assays, and molecular markers of drug resistance (WHO, 2009; Nsanzabana *et al*., 2018). Molecular markers of drug resistance are essential tools for large-scale surveillance studies of resistant *Plasmodium* parasites worldwide. The advantage of molecular markers of drug resistance is that this method can be more easily standardized and rapidly deployed as compared to the *in vivo* and *in vitro* methods for monitoring resistance. It is also cost-effective in routine surveillance and not impacted by host immunity (Nsanzabana 2021). It involves the detection of mutations in the *Plasmodium* parasite genomic DNA when amplified through Polymerase Chain Reaction (PCR) techniques and sequenced. Meanwhile, the *in vivo* method involves the monitoring of *Plasmodium* parasites clearance rate within the blood of an infected individual over a given period after the administration of a standard dose of antimalarial. This method allows for the concurrent assessment of the patients, parasites, and drugs, thus providing information about the drug efficacy in patients. However, the result of *in vivo* test could be affected by both patient's immunity and malabsorption. Additionally, it is highly expensive and involves lots of logistics (WHO, 2009). Concerning the *in vitro* method, it is employed

in the concurrent assessment of more than one drug and for more than one parasite. It involves the artificial culturing of parasites against different drug concentrations (WHO, 2001).

The World Health Organization's Global Technical Strategy 2016-2030 has set the goal to reduce malaria by 90% by 2030 (WHO, 2015). Thus, to align with this set goal, monitoring malaria transmission and *P. falciparum* drug resistance in all malaria-endemic zone, particularly Nigeria is highly important. Therefore, this study will focus on the epidemiology of falciparum malaria transmission, diagnosis, and the molecular surveillance for antimalarial drug resistant gene markers in Akure, Nigeria.

## 1.2 Problem statement

Malaria remains a major public health problem worldwide, particularly in tropical Africa. Accurate and prompt malaria diagnosis remains a major problem in the control and elimination of malaria disease, particularly in malaria endemic resource-poor settings including Nigeria. The microscopy technique which is considered the gold standard and RDT remains the major tools for malaria diagnosis in most malaria-endemic regions including Akure, Nigeria. However, studies have shown that the performance of these tools are reducing gradually, thereby leading to underdiagnosis, overdiagnosis, and misdiagnosis. In turn, this could lead to undertreatment, mistreatment, and overtreatment which can ultimately influence the development of drug-resistant parasites strains accompanied by untold havoc on the public health at large. Therefore, it is essential to determine the prevalence of *Plasmodium* species in Akure using RDT and microscopy examination in comparison with Polymerase Chain Reaction (PCR) in order to determine the accuracy and performance of these tools, since PCR is highly sensitive and specific. This will help

to make informed guidelines and policy regarding the best diagnostic tool to be employed locally in Akure, Nigeria.

Moreover, malaria continues to remain a major public health challenge since the baseline data on the epidemiology such as prevalence, parasite density and risk factors of falciparum malaria upon which control could be based and managed is largely unknown and inadequate in Akure, Nigeria. Therefore, there is a need for the epidemiological baseline data update for falciparum malaria in Akure, Nigeria. This is very essential in order to enable and guide a timely and effective malaria control and prevent outbreaks of malaria disease.

Despite all the control measures and interventions, malaria infection remains unabated. An important factor for the persistence of malaria infection is the emergence of drug resistance. Detailed and updated epidemiological baseline records for falciparum malaria and early detection of emerging drug-resistant falciparum malaria can provide an opportunity for proactive control interventions. Currently, *pfcrt* and *pfmdr1* have spread to Nigeria. Moreover, there is a growing concern that artemisinin-resistant falciparum malaria (marked by *Kelch13* mutation) which was found in Cambodia, Thailand, and Vietnam may spread to or even emerge locally in sub-Saharan Africa including Nigeria. The consequences can be a grave implication for public health as it may lead to millions of deaths worldwide, particularly in Nigeria. Currently, molecular surveillance data for drug-resistant falciparum malaria is lacking in Akure, Nigeria. Thus, there is a need to carry out the molecular surveillance of drug-resistant falciparum malaria in Akure, Nigeria in order to determine their prevalence and role. This is highly important as guides to prevent local emergence, reemergence, and spread of falciparum resistant strains, to develop drug and updating guidance for drug policy (whether a particular drug should be used or not), and to track progress in

malaria control. Without this epidemiological information on drug resistance, the control and containment of malaria parasites may only be a mirage.

## 1.3 Research objectives

The research objectives of this study are:

1. To identify the *Plasmodium* species that are prevalent in Akure, Nigeria, using rapid diagnostic test (RDT), light microscopy and nested polymerase chain reaction (nested PCR), and to compare between the diagnostic accuracy and performance of these selected tools.
2. To determine the prevalence, parasite density and risk factors of falciparum malaria with respect to demographic factors, socioeconomic factors, environmental factors, ABO blood groups, haemoglobin genotype, and knowledge of participants about malaria in Akure, Nigeria.
3. To determine the prevalence and genetic polymorphism of *Plasmodium falciparum kelch13 (pfk13)* propeller mutation, *Plasmodium falciparum chloroquine resistance transporter* (*pfcrt*) gene, and *Plasmodium falciparum multidrug resistance 1 gene (pfmdr1)* in Akure, Nigeria.

# CHAPTER 2

# LITERATURE REVIEW

## 2.1 Malaria

### 2.1.1 Malaria parasites

The history of malaria is dated back to the antiquity as it was first discovered in the human blood by a French military doctor in France Health Service of the Armed Forces, Charles Louis Alphonse Laveran on November 6, 1880 (Russell, 1965; Bruce-Chwatt, 1981; Cox, 2010). Laveran observed spherical bodies that are adherent to the red blood cells which were glassy or hyaline, thus difficult to observe. He also observed other bodies that contained dark granules of pigment exhibiting ameboid movements and others that were crescent-shaped. Surprisingly, he finally observed a parasite within the red blood cell of a malaria-infected patient who have been febrile for 15 days at a hospital in Constantine, Algeria while working as a military doctor.

Malaria is caused by an obligate intracellular protozoan parasite of the genus *Plasmodium* belonging to the subclass Coccidia and phylum Apicomplexa (WHO, 2015). Though there are several species of *Plasmodium*, only five species are recognized to infect, cause symptoms and lead to severe malaria complications in humans namely *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium knowlesi* (Caraballo, 2014; WHO, 2020).

*Plasmodium falciparum* (Figure 2.1) is well known as the most prevalent, virulent and pathogenic species of *Plasmodium* (WHO, 2015). In Africa, *P. falciparum* accounted for an estimated 99.7% of all malaria cases in 2018 (WHO, 2017). Similarly in 2019, *P. falciparum* malaria was responsible for an estimated 99% of all malaria cases in Africa as well as approximately 94% of all malaria cases and deaths

worldwide (WHO, 2020). It causes the most dangerous form of malaria infection known as falciparum malaria which mostly results to severe malaria such as cerebral malaria if not treated on time. Thus, almost all malaria deaths are associated with *P. falciparum*. It has been revealed that *P. falciparum* trophozoites and schizonts forms have the capacity to sequester in the deep venous microvasculature. *Plasmodium falciparum* sequestration occurs when the infected erythrocytes adhere to the endothelial cells and when there is rosetting which is the binding of infected erythrocytes to uninfected erythrocytes. The name, *P. falciparum* was created by Williams H. Welch in 1897 and was formally adopted by the International Commission on Zoological Nomenclature (ICZN) in 1954. *Plasmodium falciparum* life cycle has been widely studied and has been observed to possess various forms of developmental stages during its life cycle (Figure 2.1). Furthermore, it has been revealed that asymptomatic *P. falciparum* malaria may remain for up to a decade or even more with the highest number of years established to be 13 years (Ashley and White, 2014). This has further strengthened the fact that *P. falciparum* remains the deadliest malaria parasite.

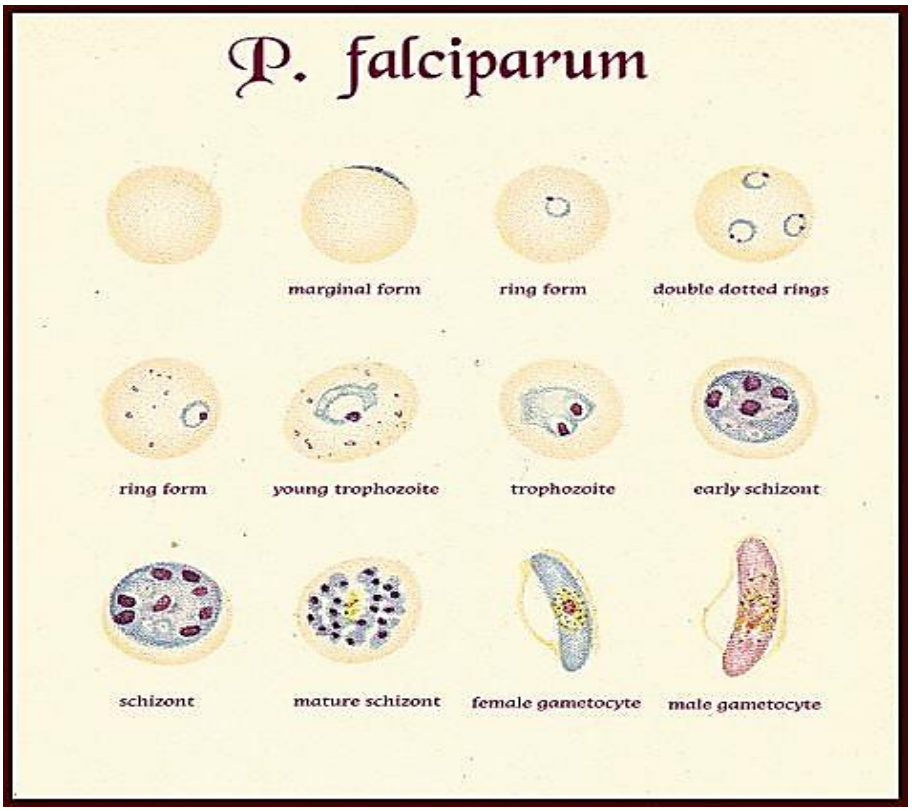


**Figure 2.1** *Plasmodium falciparum* developmental stages such as the ring form, young trophozoites, trophozoite, early schizont, schizont, mature schizont, female gametocyte and male gametocyte (CDC, 2011).

Regarding *P. vivax* (Figure 2.2), It is the most widespread geographically worldwide, particularly in Southeast Asia, Central and South America, Pacific highlands, and sub-Saharan Africa (Howes *et al*., 2016; Yman *et al*., 2019; Price *et al*., 2020). In 2020, *P. vivax* alone accounted for approximately 2% (4.5 million) of all malaria cases worldwide (WHO, 2021). Unequivocally, *P vivax* have been demonstrated and regarded as the malaria parasites that can survive for many years in human host as a result of their latent or hiding forms known as hypnozoites which remain dormant in the liver for decades.

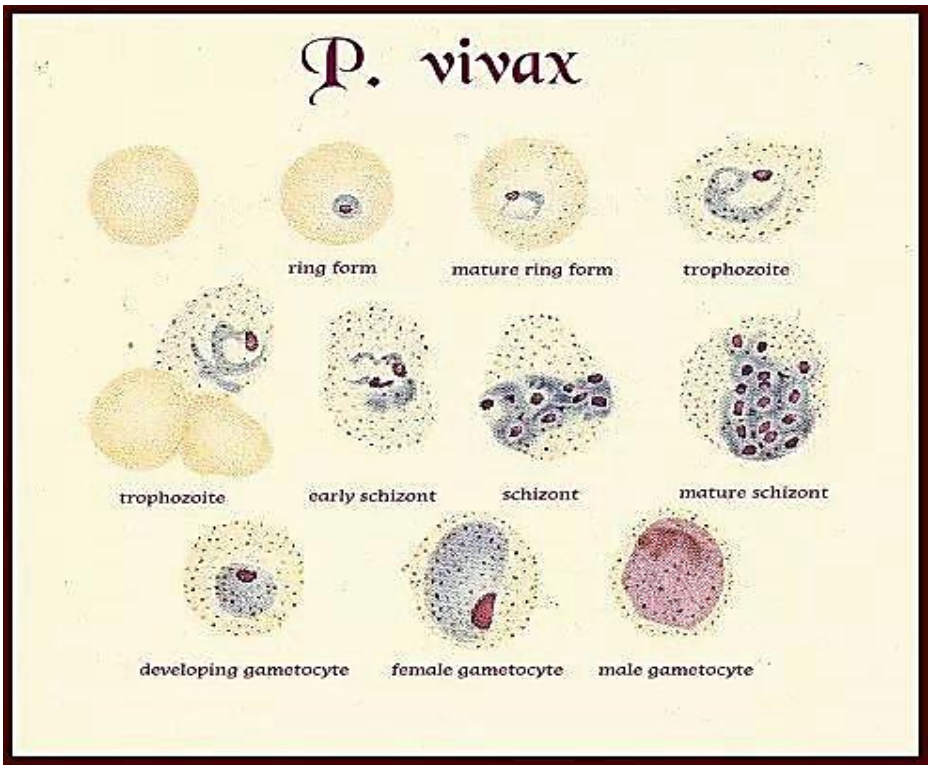


**Figure 2.2** *Plasmodium vivax* developmental stages such as the ring form, young trophozoites, trophozoite, early schizont, schizont, mature schizont, female gametocyte, and male gametocyte (CDC, 2011).

As for *P. malariae* (Figure 2.3), it is believed to significantly have lower prevalence worldwide compared to other malaria species (Barnadas *et al*., 2007). Also, infection with *P. malariae* is usually asymptomatic with low density and mild clinical outcome which may become chronic, and as such rarely lead to severe condition (Maguire and Baird, 2010; Sutherland *et al*., 2016; Yman *et al*., 2019). It is also known as quartan malaria since it is the only malaria parasite that causes fever that reoccur periodically between the interval of three days which is prolong than the two-day (tertian) intervals of the other malaria parasites (Bartoloni *et al*., 2012). It has the longest incubation period which spans between 18 to 40 days or even longer (Warrell 2002). *Plasmodium malariae* has been confirmed to be endemic in some parts of Africa including Nigeria (Dinko *et al*., 2013; Doderer-Lang *et al*., 2014; Oboh *et al.,* 2018; Roman *et al*., 2018; Amoah *et al.,* 2019), Central and South America (Bardach *et al*., 2015; Camargo-Ayala *et al*., 2016; Alho *et al*., 2017; Niño *et al*., 2016), and Asia (Nurdin *et al*., 2003; Vythilingam *et al*., 2005; Mueller *et al*., 2005; Mueller *et al*., 2007; Yavne *et al*., 2017; Roman *et al*., 2018). *Plasmodium malariae* which does not have any latent form can remain without symptom in the blood for decades possibly throughout the entire lifetime of the host (Ashley and White, 2014).

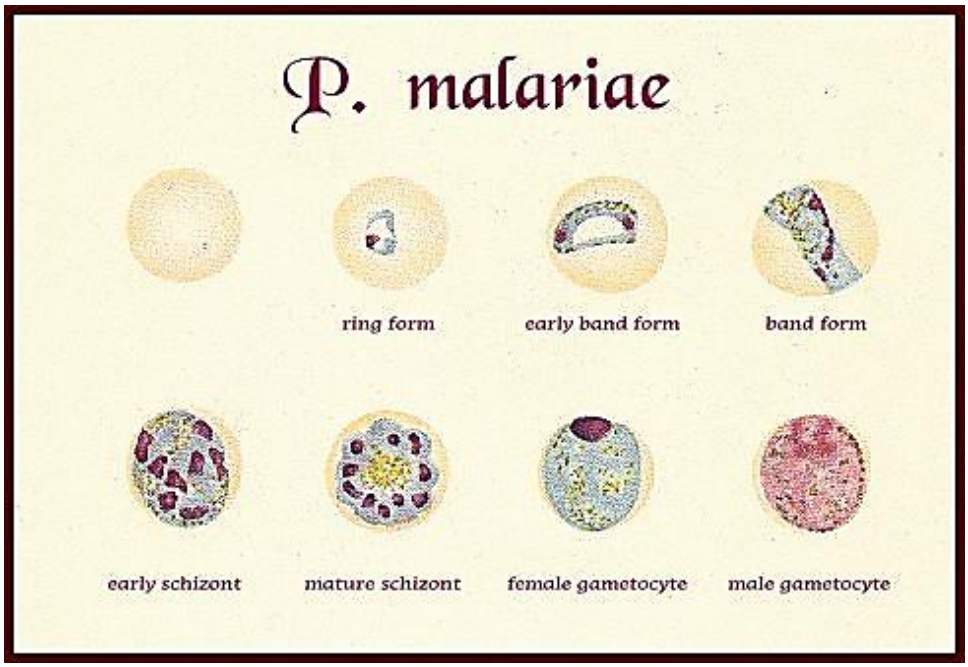


**Figure 2.3** *Plasmodium malariae* developmental stages such as the ring form, young trophozoites, trophozoite, early schizont, schizont, mature schizont, female gametocyte and male gametocyte (CDC, 2011).

Regarding *Plasmodium ovale* (Figure 2.4), it has the latent form known as hypnozoite similar to *P. vivax* and can thus remain hidden in the host for a long period of time. Unequivocally, *P. ovale* has been found to have two subspecies which include *Plasmodium ovale curtisi* and *Plasmodium ovale wallikeri*. *Plasmodium ovale* is known to have a very low prevalence with limited distribution across the world. It has been confirmed endemic in a few places such as tropical Africa, Indonesia, Philippines, India, and Southeast Asia (Collins and Jeffery, 2005). Moreover, Hawadak *et al*. (2021) revealed in a recent review and meta-analysis that the prevalence of *P. ovale* was as low as 0.77% and mostly occurred as a coinfection with other *Plasmodium* species, particularly in African regions. Furthermore, Zhang *et al*. (2021) reported that both *P. ovale curtisi* and *P. ovale wallikeri* were found to be simultaneously prevalent in 14 African countries and also noted that *P. ovale* species have latency periods that is greater than three years.

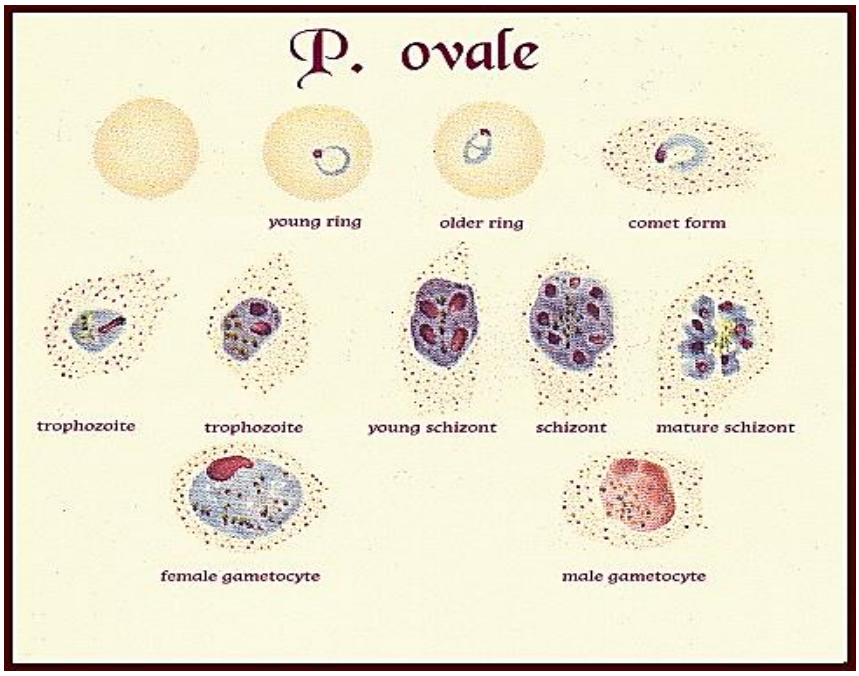


**Figure 2.4** *Plasmodium ovale* developmental stages such as the ring form, young trophozoites, trophozoite, early schizont, schizont, mature schizont, female gametocyte, and male gametocyte (CDC, 2011).

Furthermore, *P. knowlesi* (Figure 2.5) is known to cause zoonotic malaria in humans in Southeast Asia and it is generally maintained in both long-tailed (*Macaca fascicularis*) and pig-tailed (*Macaca nemestrina*) macaque monkeys (Singh and Daneshvar, 2013; Cooper *et al*., 2020). It could lead to severe malaria if proper care and treatment are not initiated and administered at the right time (WHO, 2013; Grigg *et al*., 2018). It was initially misdiagnosed as *P. malariae* before the advent of molecular tools for proper identification.

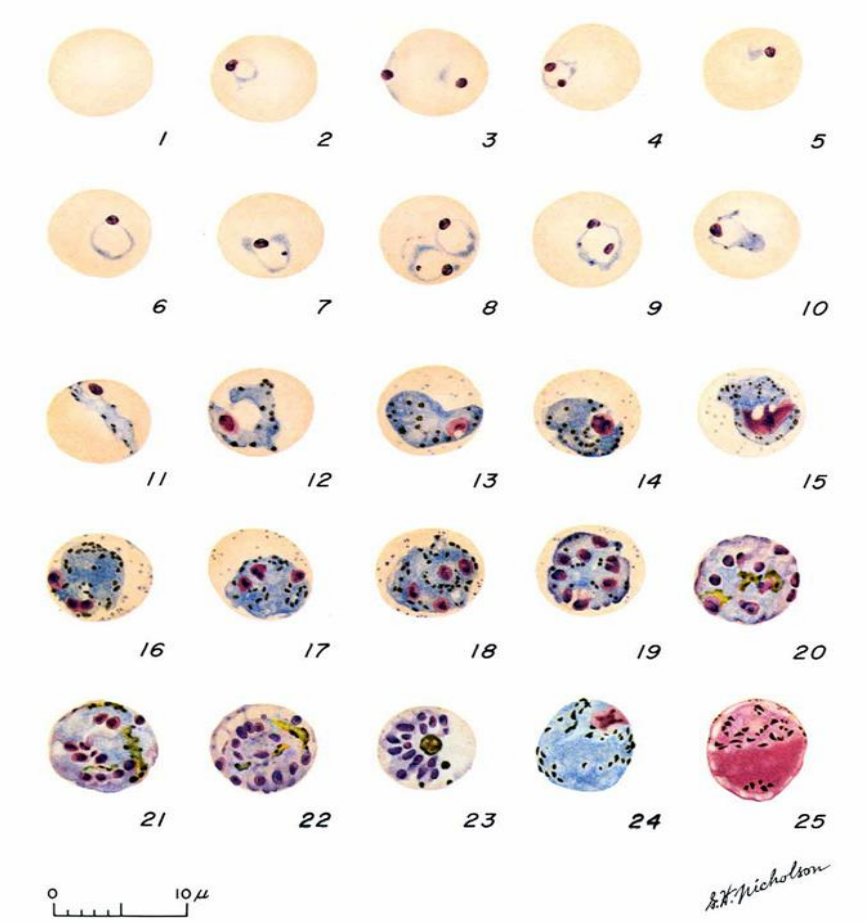


**Figure 2.5** *Plasmodium knowlesi*: 1: Normal red cell; 2-9: Young trophozoites (ring-form trophozoites); 10-12: Developing trophozoites; 13-15: Mature trophozoites; 16-23: Developing, nearly mature and mature schizonts; 24: Mature macrogametocyte (female); 25: Mature microgametocyte (male) (Coatney *et al*.,1971).

### 2.1.2 Life cycle of malaria parasites

The life cycle starts when a *Plasmodium*-infected female *Anopheles* mosquito bites a human host during blood feeding thereby injecting sporozoites with its saliva into the bloodstream of the human host through the skin. About 20 - 30 sporozoites are usually injected into the human host during a single bite and this is sufficient to cause malaria infection in humans (Satoskar, 2009). The sporozoites move through the bloodstream and leave the blood vascular system to infect the liver cells (hepatocytes) within approximately 30 minutes to begin the exo-erythrocytic cycle. The sporozoites multiply asexually through schizogony to form schizont in the liver cell (hepatocytes) within 5 - 7 days without any clinical symptoms in an infected individual. These schizonts further mature and rupture thereby releasing merozoites into the red blood cells (erythrocytes) to begin the erythrocytic cycle. This indicates that the exo-erythrocytic cycle has come to end.

A liver schizont contains thousands of merozoites which could be between 2,000 to 40,000 merozoites, depending on parasite species. The released merozoites thus rapidly penetrate and invade the red blood cell (erythrocytes) and undergo another asexual development called erythrocytic schizogony also known as the erythrocytic cycle. During this stage, the merozoites develop to form immature or ring stage trophozoites which later develop to mature trophozoites. Subsequently, the mature trophozoites develop into erythrocytic schizont. The number of merozoites in the mature schizonts is highest in *P. falciparum*, which is up to 36 and 24 in *P. vivax* while *P. malariae* usually releases about 6 - 8 merozoites which is the least. Thereafter, the infected red blood cell (erythrocytes) ruptures and release merozoites which can invade a new red blood cell and initiate a new cycle or the merozoites can develop into gametocyte which differentiate into male (microgametocytes) and female

(macrogametocytes). These gametocytes continue to circulate in the blood stream waiting for mosquitoes to ingest during blood meal.

The duration of the erythrocytic cycle varies with respect to the various species. The cycle is about 48 hours in *P. vivax* and *P. ovale* is about 48 hours (tertian malaria), 72 hours in *P. malariae* (quartan malaria), 36 hours in *P. falciparum* (tertian and modified sub tertian) while it is as short as 24 hours in *P. knowlesi* (quotidian malaria) (Satoskar, 2009). When a female *Anopheles* mosquito feeds on a blood meal from an infected person, it ingests the male and female gametocytes. Inside the mosquito, the microgametocytes and the macrogametocytes develop to become microgametes and macrogametes respectively. The microgametes and the macrogametes thus undergo sexual reproduction in the stomach of the mosquito. The microgametes fertilize the macrogametes to form zygotes. The zygote develops into an ookinete which is elongated and motile. The ookinetes invade the midgut wall of the mosquito where they develop into oocytes. The oocytes undergo a multiple multiplication process known as sporogony to form thousands of spindle-shaped sporozoites. The oocyst become ruptured and release the sporozoites which is the infective stage in humans. The sporozoites then migrate and invade the mosquito's salivary glands where it continues to wait to be injected into a new host during mosquito feeding (Paaijmans *et al*., 2009). This process can be completed around 10 - 18 days depending on the *Plasmodium* species and temperature (Paaijmans *et al*., 2009). The mosquito continues to remain infectious for 1 - 2 months (Liljander, 2010). The general life cycle of *Plasmodium* species is shown in Figure 2.6.

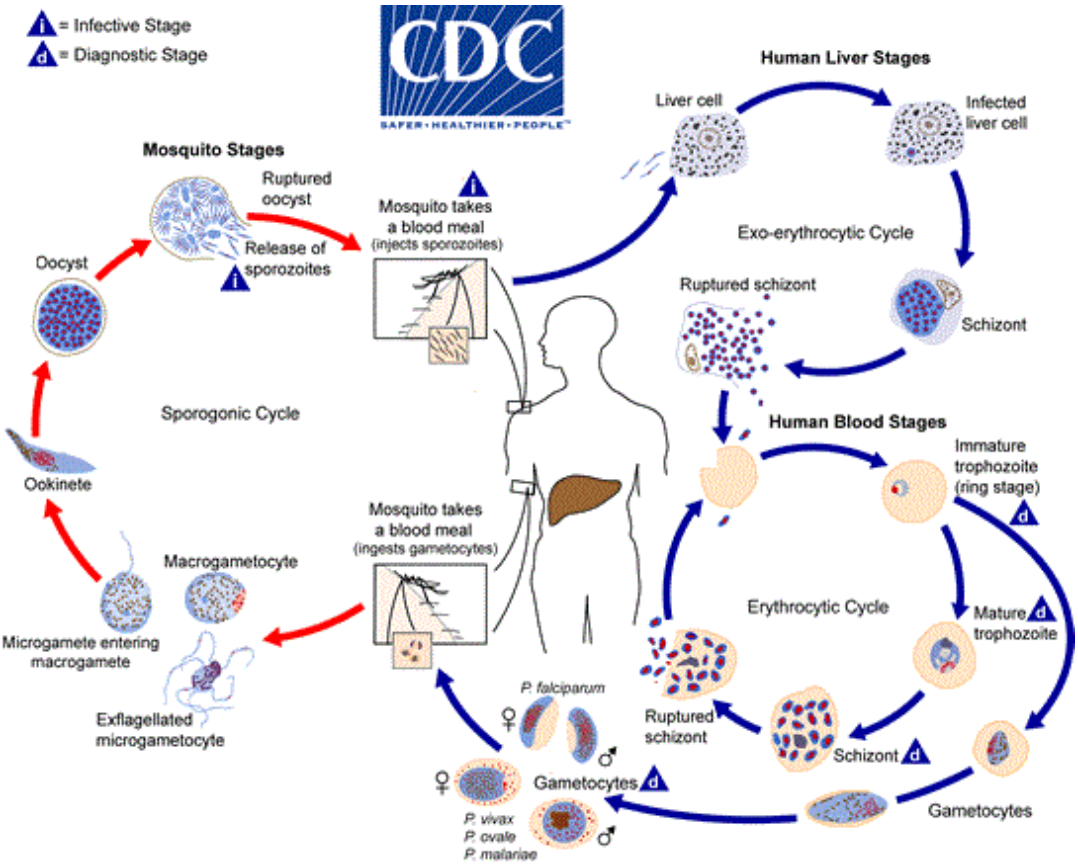


**Figure 2.6** Life cycle of *Plasmodium* species (CDC, 2017).

### 2.1.3 Mode of transmission

#### 2.1.3(a) Malaria vector

The malaria parasite is transmitted by female mosquitoes of the genus *Anopheles* belonging to the family Culicidae (Figure 2.7). The family Culicidae is made up of approximately 3500 species grouped into 41 genera. *Anopheles* mosquitoes comprise about 485 identified species worldwide of which only 41 have been associated with and have the capacity to transmit malaria at a level of major public health concern and as such regarded as the dominant vector species/species complexes (DVS) (Hay *et al*., 2010; Sinka *et al*., 2012; Harbach and Kitching 2016; Stevenson and Norris, 2017). The distribution of malaria mosquito vectors is basically dependent on climatic and environmental factors such as rainfall pattern, temperature, humidity,

and topography of the land surfaces (Balls *et al*., 2004). Consequently, malaria transmission is seasonal in many regions since malaria is mostly transmitted in the rainy season compared to the dry season. The malaria vector, *Anopheles* mosquitoes use clear and clean water as their breeding site to lay eggs which hatch and develop into larvae and eventually emerge as adult mosquitoes. The female *Anopheles* mosquitoes continue to feed on blood meals so as to nurture their eggs. Also, quite a high number of mosquitoes species inhabit both artificial and natural containers such as holes of trees, shells of coconut, leaf axils, septic tanks, bamboo stumps, pools, gutters, and septic tanks for breeding (Aigbodion and Anyiwe, 2005). Additionally, *Anopheles* mosquitoes inhabit their unique aquatic habitats such as small, shallow freshwater puddles. Generally, major habitats where mosquitoes breed in Nigeria include open ground pools (pools of water on the ground), containers (interlock mould, tomato tins, take away packs, buckets), gutters (pools of water in gutter) tyre (pools of water in disposed tyre), tyre tracks (pools of water in tracks created by tyre of moving vehicles), marshy pool (ground pools of water with grasses), and swamps (naturally occurring swamps) (Mathias *et al*., 2020).

**Figure 2.7** Female *Anopheles* mosquito (*Anopheles gambiae*) (WHO, 2017).

In Africa, the major mosquito species transmitting malaria disease are *An. gambiae*, *An. arabiensis* and *An. colluzzi* from the *An. gambiae* complex of sibling species, and *An. funestus* from the Funestus subgroup (Battle *et al*., 2012; Sinka *et al*., 2012; Coetzee *et al*., 2013; Wiebe *et al*. 2017). *An. gambiae* complex also known as *An. gambiae sensu lato* (that is in the broader sense) comprises of eight reproductively different species that are virtually morphologically indistinguishable or identical, and these encompass *An. gambiae sensu stricto (s.s.), An. merus, An. melas, An. arabiensis, An. bwambae, An. amharicus, An. quadriannulatus,* and *An. coluzzii*. Some major species of *Anopheles* complex capable of transmitting malaria include *An. arabiensis, An. coluzzii*, and *An. gambiae* (Wiebe *et al*., 2017). These are vectors of human malaria parasites with varying degrees of vectorial capacity.

Out of all the different species in the *An. gambiae* complex, *An. gambiae sensu stricto* is known to be the most dominant and efficient vector of human malaria in the Afrotropical Region (Sinka *et al*., 2010; CDC, 2015). This is due to its high abundance, diversity, longevity, vectorial capacity, and natural tendency and inclination to feed on humans (Takken and Scott, 2003; Autino *et al*., 2012). Within the *An. gambiae* species complex, *An. melas* and *An. merus* are becoming dominant and important malaria vectors capable of transmitting malaria disease in one region or more while other species have no evidence of malaria transmission (Kipyab *et al*., 2013; Ebenezer *et al*., 2016).

In recent times, *An. stephensi* (Liston) common in many Asian countries such as Thailand, India, and southern China have been reported in Djibouti City, Ethiopia, Somalia and Sudan (Sinka *et al*.,2011; Faulde *et al*., 2014; Carter *et al*.,2018; Seyfarth *et al*., 2019). To date, *An. stephensi* was detected from 46 different sites in these locations (WHO, 2021). It can transmit both *P. falciparum* and *P. vivax* parasites and

this is a great concern for African countries, which are already bearing the highest malaria morbidity and mortality worldwide. Some of the major factors affecting malaria vector distribution include climatic factor such as temperature, rainfall or precipitation and humidity (Khasnis and Nettleman 2005; Pascua *et al*., 2006; Mattah *et al*., 2017). Figure 2.8 shows the distribution of dominant malaria vector species in Africa and the world at large (Sinka *et al*., 2012).

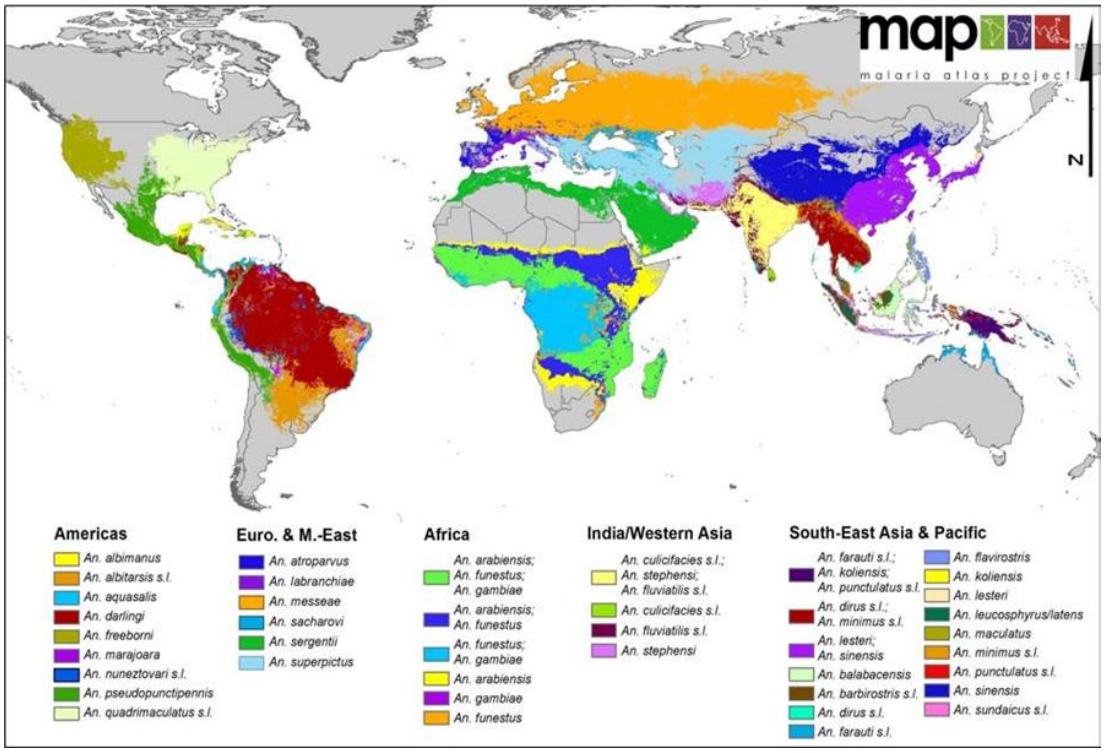


**Figure 2.8** A global map of dominant malaria vector species (Sinka *et al*., 2012).

In Nigeria, mosquitoes tend to thrive well due to conducive environmental and climatic conditions that enhance the proliferation of mosquito species. The major genera of mosquitoes observed in Nigeria encompass *Anopheles*, *Culex*, and *Aedes*. Similarly, some of the major mosquito species documented in Nigeria include *An. gambiae* complex (*An. gambiae* sensu lato), *An. gambiae* sensu stricto, *An. funestus*, *Culex pipiens pipiens*, *Cx. salinarius*, *Cx. quinquefasciatus*, *Cx. fatigans*, *Cx.*

*andersoni, Cx. restuans, Cx. nigripalpus, Cx. tigripes*, *Cx horridus, Cx. cinereux, Cx annuliorus, Aedes aegypti, Ae. Vittatus, Ae. albopictus, Eretmapodite chrysogaster* (Okorie *et al*., 2011; Mgbemena *et al*., 2012; Monsuru *et al*., 2013; Fagbohun *et al*., 2020).

As a result of the high diversity and abundance of mosquito species in Nigeria, effort has been directed toward proper control of mosquito vector in order to reduce the burden of malaria infection in Nigeria. Some of these mosquito control measures include the use of long-lasting insecticide-treated nets (LLINs), indoor residual spraying (IRS) and larval source management (LSM). Meanwhile, the most widely utilized malaria vector control intervention such as long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) are basically dependent on synthetic insecticides (WHO, 2015). Presently, organochlorines, organophosphates, pyrethroids and carbamates are the only classes of insecticides approved by the World Health Organization Pesticide Evaluation Scheme (WHOPES) to be employed in public health programs (WHO, 2017). Moreover, while dichloro-diphenyl-trichloroethane (DDT) is a major environmental hazard due to its inability to degrade and the resultant genotoxicity effect, the World Health Organization have recommended the use of dichloro-diphenyl-trichloroethane (DDT) for Indoor Residual Spray in African countries where malaria continues to be a serious health challenge, particularly when the benefits overshadow the health and environmental risk (WHO, 2006).

In Nigeria, the major class of insecticide used is pyrethroid. Meanwhile, the two major types of pyrethroid used in insecticide net treatment in Nigeria are permethrin (Type 1) and deltamethrin (Type 2) (Djouaka *et al*., 2008; WHO, 2016). In spite of these measures, mosquitoes continue to spread malaria disease at an alarming rate due to their resistance to pyrethroids which is the only class of insecticide

approved by the World Health Organization for net impregnation. The advantage of pyrethroid is that it is economical, has a fast knockdown effect, and a relatively low toxicity effect on the human population (Ngufor *et al*., 2014; WHO, 2017). Unfortunately, malaria mosquito vectors have developed resistance to this pyrethroid which is an important class of insecticide.

Resistance in mosquitoes can simply be defined as the ability of mosquitoes to survive exposure to a standard dose of insecticide due to physiological and behavioral adaptation. The emergence of insecticide resistance in a vector population is an evolutionary phenomenon due to either behavioural avoidance (such as exophily or outdoor resting instead of endophily or indoor resting) or physiological factors whereby the insecticide is metabolized by susceptible mosquitoes. As stated by World Health Organization (WHO), two-thirds of countries where malaria is endemic has developed resistance to a minimum of one class of insecticide, and Nigeria is not an exemption (Ranson *et al*., 2011). Resistance to pyrethroid by malaria vector has been reported since the year 2000 in Nigeria (Awolola *et al*., 2002). Resistance to pyrethroid by *An. gambiae* and *An. arabiensis* have now become widespread in Nigeria (Olayemi *et al*., 2011; Djouaka *et al*., 2016; Awolola *et al*., 2018; Atoyebi *et al*., 2020; Chukwuekezie *et al*., 2020). Some of this resistance involves *kdr* mutations and alterations in cytochrome P450, esterase, and glutathione-*s*-transferase (GST) at different sites (Djouaka *et al*., 2016; Awolola *et al*., 2018; Fagbohun *et al*., 2019).

### 2.1.3(b) Blood transfusion

Transfusion transmitted malaria has been documented in many parts of the world where malaria is endemic, particularly in African countries such as Nigeria (Ahmadpour *et al*., 2019). Malaria transmission through blood transfusion, which is