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Genetic and Molecular Basis for *Plasmodium Falciparum* Resistant to Antimalarian Drugs: A Review

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Abstract

Despite important gains in some areas, malaria remains a major problem in most of the tropical world, and it continue to cause hundreds of millions of illnesses and several death cases every year. Most serious illnesses and deaths from malaria and also most drug resistant infections are due to infection with *Plasmodium falciparum*, the most virulent human malaria parasite. Due to persistent lack of an effective vaccine, the fight against malaria relies mostly on chemotherapy and chemoprophylaxis. However, resistance to currently available antimalarial drugs has seriously reduced the effectiveness of the drugs and forced the scientists to start generating new and better anti-malarial drugs. It is important to understand the mechanism of the antimalarial drugs, as it is one of the key factors in the emergence and spread of drug resistance. This review aimed to summarizes the commonly used antimalarial drugs, their mechanism of action and the genetic markers validated so far for the detection of drug resistant parasites.

Keywords: *Antimalarial drugs; Malaria; Plasmodium falciparum; Resistance*

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Introduction

Malaria remains an important public health concern in countries where transmission occurs regularly, as well as in areas where transmission has been largely controlled or eliminated [1]. Malaria is considered as complicated disease due to its variation in epidemiology and clinical symptoms in different part of the world. Five species of the genus plasmodium are found to affect human. According to Olliaro [2] the 5 species include *P. falciparum, P. malariae, P. vivax, P. ovale* and *P. knowlesi. Malaria* is a vector borne disease in which the plasmodium parasites are transmitted to human through the bite of infected female anopheles mosquito [3]. The malaria continues to be a major threat to the world. In 2011, there is an estimation of more than 3.3 billion people who are at risk of malaria and most of them are associated to sub Saharan Africa [4]. According to World Health Organization (WHO), there are over 200 million cases of malaria annually in which 80% of the cases and about 90% of malaria death cases were estimated to occur in African region. The report concluded that children of less than 5 years of age and pregnant women are affected most [5]. The *plasmodium* parasite was found to be resistance to several Antimalarian drugs over a period of time and such drug resistance has emerged as one of the greatest challenges facing the control of malaria today [2].

The discovery of chloroquine (a synthetic drug) in the late 1940s has greatly helped in the treatment and eradication of malaria throughout the world especially in 1950s [6]. However, the therapeutic efficacy of CQ and efforts to eradicate malaria worldwide were diminished due to the occurrence of CQ resistance. The resistance of malaria parasite to chloroquine has led to re-emergence of the disease and rapid spread of chloroquine resistant parasite especially South America and Southeast Asia [7]. As result of lack of effective and affordable Antimalarian drug, the spread of chloroquine resistant malaria parasite to Africa in 1980s claimed 2 to 3 fold increase in death related to malaria [8]. Resistivity to chloroquine has led to introduction of sulfadoxine/pyrimethamine (SP) as the first line treatment for malaria parasite, but unfortunately, the parasite become resistant to sulfadoxine/pyrimethamine and become widely spreaded [9].

The use of antimalarial drug as a combination therapy was introduced instead of single that has been previously practiced. Combination therapy used to increase the effectiveness of the drug and to reduce the emergence of drug resistance parasite. As result, Artemisinin based Combination Therapies (ACT) have been most widely and effectively used for treatment of malaria. Recently, a report of Artemisinin resistance has been found in Southeast Asia and thus, increases the global alarm for treatment and control of malaria [10]. According to World Health Organization (WHO), resistance to anti-malarial drug is the ability of *plasmodium* parasite to survive and multiply despite the administration and absorption of a medicine given in doses equal to or higher than those usually recommended but within the tolerance of the subject. In respect to the above statement, "the form of the drug active against the parasite must be able to gain access to the parasite or the infected red blood cell for the duration of the time necessary for its normal action" [11]. There are some anti-malaria drugs that encountered plasmodium resistance very quickly after their introduction. The development and rapid spread of malaria parasite to the most commonly used anti-malarial drug is a major challenge in malaria control [4].

Mode of Action and Status of Resistance of Antimalarial Drugs

Quinolines

Quinolines are the oldest class of antimalarial drugs. The first drug in quinoline class is the Quinine. The quinine is an alkaloid derivatives isolated from the bark of *Cinchona* tree [12]. Quinine and its derivatives are in still used for the treatment of malaria and several years after its discovery, quinine has remained effective and still recommended for the treatment of severe cases of malaria and as a second line treatment in combination with antibiotics to treat resistant malaria [1]. The mechanism of action of quinoline anti-malarial drugs has not been well elucidated but quinine appears to interact with heme detoxification.

There has been a rare report of resistance to quinine, but there have an isolated cases reported from Thailand and East Africa [13]. A case of resistance to quinine was also recently reported in Northern India, where patients with severe malaria encountered a treatment failure 5 days after starting the treatment [14]. According to Saifi., *et al*. [15] the sporadic resistance to quinine by *Plasmodium* parasite might be linked to poor quality of drugs or inadequate treatment rather than the Plasmodium parasite resistance.

Chloroquine CQ is another derivative of quinine that was first synthesized in 1934 and introduced as the drug of choice for the treatment of uncomplicated and non-severe malaria and for chemoprophylaxis in the 1950s [1]. Unfortunately *Plasmodium falciparum* has developed resistance to chloroquine and the spread of resistance prompted the change in policy and removal of CQ from antimalarial therapies. It is mostly accepted that CQ kills malaria parasites by interfering with the detoxification of ferriprotoporphyrin IX (FP), a heme metabolite, in consequence causing it to accumulate to lethal levels.

FP is produced when the parasites denature or degrade hemoglobin. It is detoxified by polymerization to the crystal-like hemozoin [16]. Resistance to CQ is known to be associated with a parasite protein named CQ-resistance transporter, *PfCRT*, and the mutated form of the pfcrt gene is able to reduce CQ accumulation in the digestive vacuole of the pathogen. Additional mutations on the multidrug resistance gene 1 (*pfmdr 1*) are also associated with resistance to CQ. Despite the widespread resistance, CQ remains an efficacious drug for the treatment of vivax malaria in Afghanistan [17].

In 1960, Amodiaquine (AQ) was developed to counteract resistance to CQ [18]. AQ and its slowly eliminated active metabolite desethylamodiaquine (DEAQ) are structurally related to CQ, this explains the cross resistance observed in the field, where parasites where reported to harbor mutations on *pfcrt* and *pfmdr1* after AQ treatment failure [19]. AQ is currently recommended to be used in combination with artesunate for the treatment of malaria [20].

Mefloquine (MQ) is another widely used quinoline drug, developed in the 1970s as a strategy to counteract resistance to CQ. MQ is currently recommended to be used in combination with artesunate for the treatment of uncomplicated falciparum malaria especially in regions of multidrug resistance like South East Asia [1]. Resistance to MQ has been reported and studies suggest that the copy number of pfmdr-1 is associated with the observed resistance [21].

Piperaquine (PPQ) is a bisquinoline antimalarial drug developed in the 1960s in China [22] in response to the increasing prevalence of CQ-resistant parasites in Southern China. PPQ was adopted as the first-line treatment in 1978 [23]. Its application as monotherapy, however, resulted in the eventual emergence of PPQ-resistant parasites, which diminished its use by the late 1980s [23]. PPQ was subsequently combined as part of China-Vietnam 4 (known as CV4), an ACT that achieved high cure rates and that consisted of dihydroartemisinin (DHA), trimethoprim, PPQ, and primaquine (PQ) [22]. This combination has been revised, and PPQ is currently recommended by the WHO to be administered in combination with DHA. This combination has undergone successful clinical evaluation in both Africa and Asia [24]. The mechanism, by which resistance is mediated, however, remains unclear. PPQ resistance was recently reported to be associated with a copy number variation on chromosome 5 (that includes pfmdr1) in drug-pressured P. falciparum parasites [23].

PQ is an 8-aminoquinoline approved for the treatment of malaria since 1952 by the Food and Drug Administration (FDA) [25]. It is one of very few medications active against the liver stages of Plasmodium. Furthermore, PQ has long been reported to have potent activity against the mature gametocytes of P. falciparum [25]. These stages are able to continue the parasite life cycle in the mosquito, once taken up during a blood meal, and thus play an essential role for malaria transmission from human to human. In order to block transmission of resistant gametocytes, current WHO guidelines recommend the addition of a single dose of PQ to ACT for uncomplicated *falciparum* malaria as a gametocytocidal compound, particularly as a component of a pre-elimination or an elimination program [1]. Results from a recent clinical trial suggest that addition of single-doses of PQ shortens the infectivity period of DHA-PPQ treated patients and should be considered in low-transmission regions that aim to control and ultimately eliminate *falciparum* malaria. Resistance to PQ is a difficult entity to quantify, because PQ is not used in isolation, it is combined with a blood schizontocidal agent, and the lack of efficacy between the two drugs is difficult to quantify separately [25].

Lumefantrine also named benflumetol is an aryl alcohol, first synthesized in the 1970s in China and registered in China for the treatment of malaria in 1987 [26]. It is the only compound of this class approved for the treatment of malaria. In contrast to most other ACT partner drugs, Lumefantrine has never been used or recommended as monotherapy. It is used in combination with artemether as the first-line treatment for uncomplicated malaria. Resistance to Lumefantrine in field isolates has not yet been convincingly demonstrated.

Anti folates

Simultaneously, the anti folates including proguanil and sulfadoxine-pyrimethamine (Fansidar), introduced in 1948 and 1967 [27] respectively, exert their anti-malarial actions against both *P. falciparum* and *P. vivax* by targeting two critical enzymes, dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) in the folate pathway [28]. Proguanil, abiguanide derivative and a product of British antimalarial research, targets DHFR. DHFR converts dihydrofolate to tetrahydrofolate. On the other hand, sulfadoxine-pyrimethamine, combined together due to their synergistic mechanism of action, target both DHFR and DHPS. DHPS is a key instrument in the synthesis of dihydropteroate, which precedes folate synthesis. The folate pathway assumes significance as the products of this pathway, i.e. purines, Pyrimidines and amino acids are indispensable for the growth and propagation of the parasite [28].

Further, the stability in the differences in the amino acid sequences between the parasite and the humans allow a more parasite targeted action. Unfortunately, the effectiveness of this class of drugs diminished rapidly due to development of resistance within a year of their introduction [27]. The molecular basis of anti-folate resistance has been extensively studied and documented. A handful of point

mutations at the codons leading to various amino acid sequence changes have been implicated behind anti-folate resistance [28]. Resistance to proguanil was observed as early as the 1950s. As noted, A16V (alanine to valine at codon 16), S108T/N (serine to threonine at 108 codon) or N51I (asparagine to isoleucine at 51 codon), C59R (cysteine to arginine at 59 codon), I164L (Isoleucine to leucine at 164 codon) and S108N (Serine to asparagine at 108 codon) mutations have been elucidated to being responsible for production of mutant DHFR in the parasite, thus rendering proguanil ineffective [28].

Similarly, resistance against pyrimethamine and sulfadoxine, noted first in the mid-1970s, has been registered by virtue of production of mutant DHFR enzyme due to N51I, C59R, S108N, I164L or C50R (Cysteine to asparagine), N51I, S108N, I164L mutations [28]. Further, S436A/F (serine to alanine at 436 codon), A437G (alanine to glycine at codon 437), K540E (lysine to glutamic acid at codon 540), A581G (alanine to glycine at codon 581), A613S/T (alanine to serine/threonine) point mutations have led to the production of mutant variety of DHPS [28]. This combined with mutant variety of DHFR enzyme, further aggravated the resistance against sulfadoxine-pyrimethamine in the subsequent years. Due to widespread resistance against it, pyrimethamine-sulfadoxines no longer recommended for the treatment of uncomplicated malaria or for chemoprophylaxis. However, it is still being recommended for the intermittent preventive treatment of malaria in pregnancy in malarious areas. Further it is being evaluated for the same in infants [29]. Proguanil as a single agent, on one hand, has been found to be effective against chloroquine and pyrimethamine-sulfadoxine resistant strains of *P. falciparum* found in sub-Saharan Africa. However, on the other hand, it has been reported to be ineffective against multidrug-resistant strains of *P. falciparum* in Thailand and New Guinea. In the wake of widespread resistance against proguanil, it is now being utilized in combination with atovaquone, especially in areas where highly drug resistant strains of *P. falciparum* are rampant. Resistance against this combination is highly uncommon, unless the parasite specie was resistant to atovaquone in the first place.

Artemisinin and Derivatives

Artemisinin is a potent and rapidly acting blood schizontocide, which is active against all Plasmodium species. Artemisinin was originally isolated from the plant Artemisia annua, an herb employed in Chinese traditional medicine. It has an unusually broad activity against asexual parasites, killing all stages from young rings to schizonts. In view of the alarming emergence of chloroquine resistance among malarial parasites, Project 523 was initiated in 1967 by the Chinese government. This ultimately yielded in Artemisinin and its derivatives, artesunate and artemether, which are now preferred drugs in all chloroquine resistant areas [30]. Resistance against Artemisinin, a long existing enigma in the treatment of malaria, has also been reported since 1980s [27]. The menace of Artemisinin resistance, limited initially to a few sporadic cases in the greater Mekong region, has now assumed significant proportions [31,32].

Artemisinin, a sesquiterpene lactone end peroxide contains a peroxide bridge which is responsible for its enigmatic mode of action. One line of thought dictates that the cleavage of the peroxide bridge in the presence of ferrous ions gives rise to highly reactive free radicals, which in turn lead to the parasite's death [33]. A second line of thought proposes that an interaction between Artemisinin and *PfATP6* is essential to the parasitic death [34]. *PfATP6* is the only SERCA-type (Sarcoplasm endoplasmic reticulum calcium channel) Ca21-ATPase present in the malaria parasite. Physiologically, *PfATP6* serves the same purpose as any mammalian SERCA. Studies have illustrated that Artemisinin exerts its action via *PfATP6*. Inhibition of this enzyme subsequently inhibits the action of Artemisinin.

Once inside the parasite cell, Artemisinin gets activated by free iron neighboring PfATP6 in the endoplasmic reticulum. In the presence of ferrous ions in the DV, the cleavage of the peroxide bridge occurs, producing highly reactive free radicals and ultimately leading to the parasite's death [3]. There is no established mechanism behind the development of Artemisinin resistance. However, ongoing research has identified mutations in the genes encoding for PfATP6 and amplification in the Pfmdr-1 encoding gene to being responsible for artemisinin resistance [27].

Further among the recent advances made, it has been observed that point mutation, i.e.C580Y in the propeller region of kelch motif containing gene or K13 is the prime reason for Artemisinin resistance observed in the greater Mekong region. The C580Y mutation reduces poly ubiquitination of *P. falciparum phosphatidylinositol-3-kinase (PfPI3K)* which in turn limits the proteolysis of PfPI3K leading to an increase in its level along with the level of its lipid product *phosphatidylinositol-3-phosphate (PI3P)*. Thus along with the K13

mutation marker, PI3P levels can also be predictive of Artemisinin resistance [35-38]. Despite their rapid activity and high potency, even in the face of multi-drug resistant malarial parasites, the Artemisinin are not used as a monotherapy due to their limited ability to eradicate the infection. Their short plasma half-life limits its utility as a chemo-prophylactic agent and also leads to more chances of treatment failures [38]. However, when Artemisinin are combined with other antimalarial/ACT (Artemisinin combination therapy) partner drugs, they lead to sustained antimalarial action. Hence ACT now form the first line of treatment for any form of malaria, especially in case of severe malaria. The current regimens employ Lumefantrine, mefloquine, amodiaquine and Piperaquine as the accompanying drugs to Artemisinin in the combination regimens [37].

Antibiotics

The antimalarial activity of selected antibiotics was known since the 1950s, with the most active compounds belonging to the Tetracycline's. Their effect on malaria parasites was later attributed to their action on a parasite organelle of prokaryotic origin, the apicoplast [6,39]. However, treatment of malaria with tetracyclines was not considered to be of important value because fever and parasite clearance were significantly slower compared to other antimalarial drugs. With the emergence of drug resistance to CQ in the early 1970, the use of antibiotics in malaria therapy was re-evaluated and the combination of tetracycline's with faster acting drugs (e.g. quinine) was increasingly used against CQ resistant *falciparum malaria* [20]. Currently, The WHO recommends the use of doxycycline, tetracycline and clindamycin in antimalarial therapy, either in combination with a rapid acting drug like Artemisinin derivatives or quinine as a second line antimalarial treatment [1].

Any of these combinations should be administered for 7 days given the slow mechanism of action of antibiotics. Almost all antibiotics with antimalarial activities target the prokaryotic ribosome of the organelle, thus blocking the apicoplast's translational machinery. Because the apicoplast has essential metabolic functions for the parasite, such as fatty acid synthesis type II, lipoic acid metabolism and isoprenoid biosynthesis, its functional inhibition by the antibiotics results in a slow (so called delayed) death of the parasite [39]. Resistances of the parasite to these antibiotics are not yet reported, probably due to the fact that most studies do not focus on this class of drugs or simply because they have not been routinely used as monotherapies to treat malaria.

Drug category	Drugs	Mode of action	Resistance	Resistance genes
Quinolines	Quinine	Inhibits FP detoxification	1910	Pfmdr1
	Chloroquine	Inhibits FP detoxification	1957	Pfcrt, Pfmdr1, Pfmrp
	Amodiaquine	Inhibits FP detoxification	1996	Pfmdr1
	Mefloquine	Inhibits FP detoxification	1997	Pfmdr1
	Piperaquine	Inhibits FP detoxification	1989	Pfmdr1
Aryl alcohol	Lumenfentrine	Inhibits FP detoxification	NR	-
Antifolate	Proguanil	Inhibits the dihydrofolate reductase	1952	Pfdhfr
	Sulfadoxine/Pyrimethamine	Inhibit folate metabolism	1967	Pfdhps
Naphthaquinone	Atovaquone	Mitochondrial Electron transport	1996	Pfcytb
Artemisinin	Artesunate		2006	Pfk13
	Artemether		2006	Pfk13
Antibiotics	Tetracycline/Doxycycline	Inhibits Apicoplast functions	NR	-
	Clindamycin	Inhibits Apicoplast functions	NR	-

Table 1: *Antimalarial drugs mode of action and molecular markers for drug resistance.*

Conclusion

Emergence and spread of antimalarial drug resistance constitute a major threat toward the treatment of malaria. Till date, drug resistance has been reported for *P. falciparum.* Antimalarial combination therapy targeting different mechanism of action could prolong the emergence and spread of drug resistant parasites. Understanding the site of action and mechanism of the antimalarial drugs is an important tool to identify drug resistant marker, to prevent the development of drug resistance further and in the development of new antimalarial drugs. Hence, continuous monitoring and surveillance of drug resistant molecular markers in malaria endemic regions is important in determining and assisting an effective drug policy for malaria treatment. Therefore, it is recommended that, more research is necessary to find new antimalarial drugs for multidrug resistance parasites and in identification and validation of genetic markers for multidrug resistance, thereby containment and treatment of malaria can be achieved effectively.

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