Malaria has been one of the most lethal parasitic diseases worldwide. Anti-malarial drugs are one of the important tools used for the treatment and prevention of malaria infection that affect millions of people every year all over the world. Rapid spread of anti-malarial drug resistance against *Plasmodium falciparum* and use of ineffective anti-malarial drugs continuously also has become a major hurdle in the successful treatment of the malaria infection. At present, the biggest concern regarding malaria all over the globe is to treat patients with safe and effective drugs and also to avoid the emergence of drug resistant malaria parasites against currently used efficient drugs including ACTs. These review summaries the different aspects of anti-malarial drugs which are following.

### 2.1 Life cycle of *Plasmodium*

Malaria parasites spread by infecting through two hosts, female *Anopheles* mosquito act as the definitive host while human beings the intermediate host. *Plasmodium* development starts in *Anopheles* with the ingestion of the parasite by the mosquito and at the same time *Anopheles* mosquito injects sporozoites into the blood stream of malaria’s next victim. After ingestion the male gametocyte undergoes successive nuclear divisions to form eight male gametes which are liberated from the microgametocyte by the process called exflagellation. This process is induced by a drop in temperature, an increase in carbon dioxide, and mosquito metabolites like xanthourenic acid, which is also known as mosquito-derived exflagellation factor (MEF). The microgamete now fertilizes a female gamete known as macrogamete to form zygote. This zygote elongates to form ookinete within 24 hours of infection. These ookinetes are motile entities in parasite life cycle which penetrate the midgut epithelium and rapidly transform into immotile oocysts under the basal lamina of the midgut. Thousands of haploid sporozoites are formed in each oocyst after growth and multiple meiotic divisions of 10–12 days and released into the hemolymph. The hemolymph circulation migrates sporozoites to the salivary glands of mosquito, where they are released into the saliva during subsequent blood feeding and continue the cycle of transmission back to man.

The asexual phase of parasite development commences in the human host post bite with the infective mosquito. The sporozoites are lodged in the skin when mosquito bites. The
sporozoites then glide in a corkscrew movement pattern and enter the circulatory system (Amino et al., 2007). Within 30-60 minutes, the sporozoites reach liver and invade in Kupffer cells and hepatocytes (liver cells). After invading the hepatocyte, the parasite undergoes an asexual replication and this leads to the beginning of the liver stage growth, called exo-erythrocytic (or pre-erythrocytic) schizogony (Frevert et al., 2005). Inside the hepatocyte the sporozoites undergo multiple rounds of division and develop into a schizont containing thousands of merozoites. The exo-erythrocytic stage is a clinically silent phase with no symptoms and this is different for all four species i.e. it lasts for 5.5-7 days in *P. falciparum*, 6-8 days in *P. vivax*, 9 days in *P. ovale* and 14-16 days in *P. malariae* (Vaughan et al., 2008). Merosomes are the merozoites that developed in the hepatocytes and exit the liver in host cell derived vesicles which protect the hepatic merozoites from phagocytosis by Kupffer cells. The merozoites are eventually released into the circulatory system to start the erythrocytic stage of parasite development (Sturm et al., 2006; Baer et al., 2007).

In case of *P. vivax* and *P. ovale*, a proportion of the liver stage parasites remain dormant for months in the liver, known as hypnozoites, which after a latent period develops into schizonts resulting in relapse. Relapse is used to describe as the reactivation of the infection via hypnozoites while recrudescence is a phenomenon in which parasitemia falls below detectable levels and then later increases to a patent parasitemia. Merozoites released from the infected liver cells enter into the bloodstream where they rapidly invade the red blood cells. The merozoites recognize some specific proteins on the surface of the erythrocyte i.e. reticulocyte binding like protein family (RBL) and duffy binding like erythrocyte binding protein (DBL-EBP) family and actively invade the cell through receptor- ligand interactions which are mediated by various parasite proteins which include Merozoite surface proteins (MSP) and Apical membrane antigen-1 (AMA-1) (Rayner et al., 2001; Miller et al., 2002; Goel et al., 2003; Mitchell et al., 2004).

The parasite undergoes different developmental stages during the erythrocyte cycle i.e. ring, trophozoite and schizont. The young trophozoite is often called a ring form due to its morphology in Geimsa-stained blood smears. As the parasite increases in size this 'ring' morphology disappears and it is called a trophozoite. During the trophic period, the parasite ingests the host cell cytoplasm and breaks down the hemoglobin into amino acids. A by-product of the hemoglobin digestion is the malaria pigment or hemozoin. Nuclear division
marks the end of the trophozoite stage and the beginning of the schizont stage. At the end of each cycle the infected RBCs rupture and release fresh batch of merozoites that in turn infect more RBCs. It is during the erythrocytic cycle that the clinical symptoms appear in the patient. After entering the erythrocyte the parasite replicates asexually to attaining a high parasite burden and destroys each RBC that it infects and leads to the clinical symptoms of malaria.

The erythrocytic cycle is of 48hrs in case of *P. falciparum*, *P. vivax* and *P. ovale* while 72hrs in case of *P. malariae*. In *P. falciparum*, RBCs can be infected with multiple merozoites while *P. vivax* infects only young RBCs i.e. reticulocytes. During the erythrocyte stage, a subset of merozoites after infecting the fresh RBCs enter into sexual phase by either developing into male or female gametocyte, which when ingested by the *Anopheles* mosquito during blood meal undergoes the sexual phase of development. In case of *P. falciparum*, it is only the early ring stages and sexual stages that are visible in the peripheral blood and due to sequestration rest of the asexual developmental phase that takes place in the capillaries. *P. falciparum* develops small protrusions called knobs, the surface of infected RBC (iRBC). These knobs are the localization sites of various parasite derived proteins that help them in sticking to the walls of the blood vessels and also to uninfected RBCs known as cytoadherence (Baruch et al., 1995; Smith et al, 1995; Rowe et al., 1997). This cytoadherence leads to obstruction of the blood vessels which ultimately results in the dysfunction of organs including brain that leads to cerebral malaria. Thus *P. falciparum* is known to be one of the deadliest protozoan parasites, having a high mortality rate. The life cycle of *Plasmodium* is shown in Figure 4.
2.2 Anti-malarial drugs

Chemotherapy has conventionally shown an important role in the treatment and prevention of malaria infection. Most anti-malarials target the erythrocytic stage of malaria infection while pre-erythrocytic or liver stage for most anti-malarial drugs is not well characterized. Regarding all malaria species, treatment of the acute blood stage infection is necessary for effective malaria management. In case of *P. ovale* or *P. vivax* infections terminal prophylaxis is required with a drug active against hypnozoites stage, which can remain dormant in the liver for months and occasionally years in some cases, after the initial infection. Anti-malarials are classified on the basis of their anti-malarial activity and structure.
2.3 Classification of anti-malarial drugs

Anti-malarial drugs are classified on two bases i.e. anti-malarial activity depends on drug action on different developmental stages of *Plasmodium* and classification based on chemical structure which are as follow:-

### 2.3.1 Based on anti-malarial activity

**I. Tissue schizonticides** - These drugs basically act on the primary tissue form of the parasite and eliminate developing or dormant liver forms. Theoretically, tissue schizonticides prevent development of the further infection by acting on tissue stage e.g. pyrimethamine, sulphadoxine and primaquine. Primaquine drug act on the hypnozoites or dormant liver forms of parasite of *Plasmodium vivax* and *Plasmodium ovale* that cause relapse of malaria.

**II. Blood schizonticides** - Blood schizonticides are the most important drugs in anti-malarials and act on blood schizonts and prevent erythrocytic schizogony to terminate the attack of malarial fever. This class includes quinine, mefloquine, chloroquine, halofantrine, pyrimethamine, sulfadoxine etc. Fast-acting and high-efficacy blood schizonticides are also used in chemoprophylaxis treatment.

**III. Gametocytocides** - These drugs kill sexual stages and prevent transmission to mosquitoes e.g. quinine, primaquine, chloroquine and artemisinin derivatives.

**IV. Sporontocides** - These drugs make the gametocytes ineffective within the blood of the mosquito and block transmission by preventing development of oocysts in the mosquito e.g. Primaquine and chloroguanide.

### 2.3.2 Based on structure

**I. Quinoline derivatives**

- a. 4-methanoquinolones: Quinine, quinidine, mefloquine and halofantrine.
- b. 4- aminoquinolines: Chloroquine, amodiaquine.
- c. 8- aminoquinolines: Primaquine.

**II. Folate synthesis inhibitors:** Sulphones, sulphonamides, proguanil, chloroproguanil and pyrimethamine.
III. Peroxides: Artemisinin derivatives– artemether, arteether, artesunate and artelinic acid.

IV. Naphthoquinones: Atovaquone.

V. Antimicrobials: Tetracycline, doxycycline, clindamycin and azithromycin.

2.4 Common anti-malarials:

2.4.1 Chloroquine

Chloroquine is the prototype anti-malarial drug which is manufactured on a huge scale for treatment and prevention of all types of malaria infection. CQ is less expensive, safe and effective anti malarial and still in widespread use for vivax malaria. This drug can be taken both as a prophylactic and as a treatment. Chloroquine was first synthesized in Germany, but it recognized as a potent anti-malarial drug in 1940s during the Second World War. It was found to be superior synthetic anti-malarials as compared to other contemporary drugs (Coggeshall and Craige, 1949). Chloroquine became the cornerstone of anti-malarial chemotherapy for the next 40 years. It quickly became the drug of choice to treat uncomplicated *P. falciparum* infections globally, and was used as a part of the Global Malaria Eradication campaign launched by the WHO in 1955.

Chloroquine accumulates in high concentrations in the food vacuole of parasite (Geary et al., 1986a), which inhibits the parasitic enzyme and hampered the detoxification of heme into hemozoin. CQ may also obstruct the biosynthesis of nucleic acids. CQ is highly effective drug against erythrocytic forms of all species of *Plasmodium* and also for gametocytes of *P. vivax*. It rapidly controls acute attack of malaria with most patients become a febrile within 24-48 hours. It is more effective and safer than quinine for sensitive cases. CQ is readily absorbed from G.I.T, intra muscular and subcutaneous sites. Chloroquine is a relatively safer and has fewer side effects. It can cause dizziness, headache, diplopia, disturbed visual accommodation, dysphasia, nausea and pruritus of palms, soles and scalp.

2.4.2 Quinine

Quinine, chief alkaloid, derived from the cinchona bark also known as ‘Fever Bark’ was used for treating fevers in early 17th century. Quinine and cinchonine were isolated in 1820 from
cinchona by Pelletier and Caventou. Till date, quinine is obtained entirely from the natural sources due to its complex nature which is difficult to produce. Quinine is effectively used for uncomplicated and severe malaria in different therapeutic regimens of different countries. A combination of quinine, quinidine and cinchonine (Quinimax) is also used (Deloron et al., 1985). Quinine has gametocytocidal activity and acts on schizocidal stage of parasite. Quinine can be given orally, intravenously and intramuscularly. Quinine is a weaker base and it is concentrated in the food vacuoles of *P. falciparum* and act by inhibiting heme polymerase, which allows accumulation of its cytotoxic substrate known as heme. Mode of action of quinine is similar to that of chloroquine but with some differences that chloroquine causes clumping of the malaria pigment while quinine antagonizes this process (Peters, 1987). Quinine is a toxic drug and can cause renal failure, gastrointestinal symptoms like nausea, vomiting, abdominal pain, hemolysis, vertigo and night blindness. The typical syndrome of quinine side effects is called as Cinchonis, which happen due to its larger doses.

### 2.4.3 Antifolates

Sulfadoxine and pyrimethamine are the most widely used anti-malarial drugs which belong to the antifolate class. Both drugs are used in combination for the treatment of uncomplicated, chloroquine resistant *P. falciparum* malaria. Their role in malaria control is hampered by rapid emergence of resistance under drug pressure (Plowe et al., 1998). Now, SP is used in combination with artemesunate as artemisinin combination therapy (ACT) for the treatment of *P. falciparum* malaria and also used in intermittent treatment in pregnancy (IPTp).

Pyrimethamine is combined with long-acting sulfonamides which act on all stages of the asexual erythrocytic cycle and on young gametocytes except for late-stage gametocytes and hypnozoites. Fansidar (combination of SP) is valuable in curative treatment of resistant strains as well as prophylactic treatment. Pyrimethamine is safe and well-tolerated but occasionally causes skin rashes and depression of hematopoiesis. Toxicity of combination with sulfonamides is due to sulfadoxine which cause agranulocytosis, aplastic anemia, erythema multiforme of the Steven Johnson syndrome, exfoliative dermatitis, serum sickness and liver dysfunction. Pyrimethamine is completely absorbed after oral administration while sulfadoxine is rapidly absorbed from the gut. Sulfadoxine is a long acting antifolate with a
half-life of 7-9 days while pyrimethamine has a half-life of about 80-95 hours. Pyrimethamine is excreted in breast milk whereas sulfadoxine passes through the placenta freely.

2.4.4 Mefloquine

Mefloquine is a 4-quinoline methanol derivative which was discovered during the Vietnam War to protect the American soldiers from the multi-drug resistant falciparum malaria. After war, this ‘new’ drug was restricted only for multi-drug resistant falciparum malaria. It has been found that mefloquine produce swelling in the food vacuoles of parasite. The mechanism of action of mefloquine is similar to quinine wherein it forms lethal complexes with free heme that breaks membranes and also interacts with other plasmodial components. It is effective blood schizonticide recommended for the chloroquine resistant parasite. It may cause nausea, dizziness, vomiting and abdominal pain and can occur in doses exceeds than 1 g. Sometime, it can also cause arrhythmia, postural hypotension, and an ‘acute brain syndrome’ consisting of fatigue, asthenia, seizures and psychosis and it should be avoided in first trimester of pregnancy.

2.4.5 Atovaquone

Atovaquone, synthetic hydroxynaphthoquinone, has been found to be useful against the Plasmodium. It has a highly lipophilic molecule that disrupts the plasmodial mitochondrial electron transport chain at a complex III of the respiratory chain, of the protozoa. This results in the collapse of plasmodial mitochondrial membrane functions and inhibition of ATP and pyrimidine biosynthesis. It may cause fever, vomiting, diarrhea and headache.

Atovaquone plus Proguanil: A fixed combination of atovaquone and proguanil hydrochloride (Malarone™) is now suggested for both treatment as well as a prophylaxis treatment of malaria. It is highly efficacious in the treatment of uncomplicated falciparum malaria.

2.4.6 Amodiaquine

Amodiaquine is another 4-aminoquinoline derivative whose anti-malarial spectrum and adverse effects are similar to chloroquine. Previously this drug was used for
chemoprophylaxis but then it was withdrawn because of the risk of agranulocytosis (1:2000) and hepatotoxicity. Incidentally, this drug has been reintroduced in several countries not only because it is cheap but also the effectiveness for chloroquine resistant *P. falciparum* parasite.

### 2.4.7 Halofantrine

Halofantrine is a phenanthrene methanol structurally related to quinine that was developed in the 1960s by the Walter Reed Army Institute of Research and has been used as a second-line drug at that time. Its mechanism of action is unclear but may involve possible inhibition of plasmodial proton pump similar to quinine and mefloquine. Halofantrine is given orally in three 500 mg doses at a 6 hourly intervals for one day for the treatment of multidrug resistant *P. falciparum* malaria. The most common adverse effects are abdominal pain, vomiting, cough, rash, pruritus. Halofantrine use may be restricted due to its cardiotoxic side-effects and variable pharmacokinetics.

**Lumefantrine:** Lumefantrine is also a phenanthrene-methanol related to quinine, mefloquine and halofantrine. Currently, lumefantrine is used in combination with artemether in northeast regions of India.

### 2.4.8 Artemisinin derivatives

Artemisinin or Qinghaosu (“ching-how-soo”) is a sesquiterpine lactone extracted from a Chinese medicinal herb called *Artemisia annua* (sweet wormwood). In 1970, the active ingredient of this herb was isolated by Chinese scientists named as artemisinin. Artemisinin and its synthetic derivatives (artesunate, artemether, and arteether) have been used extensively in China and South East Asia and Africa, where high level of resistance has been reported against CQ and antifolate drugs (Meshnick et al., 1996). These compounds have much potential to reduce higher parasitemia than any other drug known till date (White, 1997). According to WHO, a few semi-synthetic derivatives of artemisinin known as artesunate (a water soluble ester), artemether and arteether (oil soluble preparations) have now been developed to control the multi-drug resistant *P. falciparum* malaria. Artemisinin is rapidly effective against all malaria parasites including multi resistant strains and has become most important treatment for drug-resistant malaria. The mode of action of the artemisinin drugs has not been completely resolved. The present knowledge revealed that artemisinin
makes oxygen radicals in digestive vacuole of *Plasmodium* where lots of oxygen and heme coexist and also inhibits hemoglobin proteases in presence of heme. Artemisinin has also been shown to inhibit calcium transporter *PfATPase6.*

Artemisinin and its derivatives are administered orally as well as via intramuscular route. No major clinical toxicity has been seen against this drug in thousands of people. For maximum efficacy, these compounds were used in combination with other drugs having a longer half life because when it is used as a monotherapy, it shows high incidences of recrudescence infection which leads into resistance. Artemisinin is the fastest acting anti-malarial having short half life. It is highly effective against trophozoites form of parasite and thus prevents progression of the disease and very useful in treatment of complicated *P. falciparum* malaria. Artemisinin compounds have also gametocytocidal properties which help to reduce the gametocytenogenesis, thus reducing transmission of malaria and shows high significance in preventing the spread of resistant strains. Artemether is available in injection and capsules containing 80 mg in 1 ml and 40 mg of the drug respectively. Arteether is available only as injection of 150 mg in 2 ml.

### 2.4.9 Antibiotics with anti-malarial activity

Many antibiotics have been tested for their potential anti-malarial effects. Studies for newer anti-malarials have been insufficient, thus in this context some of the antibiotics are used in malaria treatment. However, these drugs have not been able to find any place in standard anti-malarial course of therapy. Antibiotics cannot be used as single agent for the treatment of malaria instead they are given along with other anti-malarial or combinations of two antibiotics. Tetracycline and doxycycline have blood schizonticidal activity against all *Plasmodium* species, but these have no activity against any of the liver stages. In chloroquine resistant *P. falciparum* prevalent areas doxycycline (100 mg/day orally) or tetracycline (250 mg QID or 500 mg BD) can be used as a second line therapy for chemoprophylaxis of malaria. Doxycycline (200 mg/day) can be combined with quinine or artesunate for the treatment of multidrug resistant falciparum malaria. While clindamycin (900 mg TDS orally for 5 days) with quinine (650 mg TDS orally for 3-7 days) is one of the most recommended regimens for chloroquine resistant falciparum malaria.
2.5 Anti-malarial drug resistance

Anti-malarial drug resistance poses one of the greatest threats to malaria control and has played a significantly role in global malaria mortality. WHO defines anti-malarial drug resistance as “the ability of a parasite strain to survive and/or multiply despite administration & absorption of a drug given in doses equal to or higher than those usually recommended but within the limit of tolerance of the subject” (WHO, 1967). Later Bruce-Chwatt et al. modified this to include, “the amount of the drug which is active against a given parasite must be able to gain access to the parasite or the infected erythrocyte for the length of the time necessary for its natural reaction” (Bruce-Chwatt et al., 1986). Resistance to currently available anti-malarial drugs has been documented in all four species of Plasmodium (WHO, 2010b). *P. falciparum* has developed resistance to nearly all anti-malarial drugs currently in use while *P. vivax* has been found to be resistant to chloroquine and primaquine and *P. malariae* has been reported to be resistant to chloroquine and pyrimethamine in some areas (Young, 1957; Maguire et al., 2002; WHO, 2010b). *P. knowlesi*, a new malarial species that also infects humans in forest areas of South East Asia, is fully susceptible to chloroquine and other currently used drugs. The development of drug resistance poses one of the greatest threats to malaria control programme which resulted into increased malaria morbidity and mortality.

Chloroquine-resistant in *P. falciparum* was initially developed in South East Asia, Oceania and South America in the late 1950s and early 1960s. Since then, chloroquine resistance has spread to nearly all areas of the world where falciparum malaria is endemic. *P. falciparum* has also developed resistance to nearly all of the other currently available anti-malarial drugs, such as sulfadoxine- pyrimethamine, mefloquine, halofantrine, quinine and recently, a low-grade resistance to artemisinin-based drugs has emerged in South East Asia. Chloroquine-resistant *P. vivax* malaria was first identified in Papua New Guinea in 1989 and now has also been spread in South East Asia, on the Indian subcontinent and in South America (Baird et al., 2004). Oceania region vivax parasite shows higher resistance to primaquine than *P. vivax* isolates from other regions of the world.
Treatment failure is sometimes misdiagnosed as drug resistance so that a clear distinction must be made between drug resistance and drug failure. Treatment failure is not always because of drug resistance but it depends on pharmacodynamic and other factors including inaccurate dosage, poor patient compliance, drug interaction and also poor drug quality.

2.6 Development of drug resistance

Various factors such as weak immunity, high parasitemia, low malaria transmission and drug pressure lead to the occurrence and immense spread of drug resistance in malaria parasite as explained in Figure 5. Genetic mutations that confer anti-malarial drug resistance is well known for its frequent, de novo mutations, mostly single or sometimes multiple which are independent of drug effect and are considered spontaneous in nature. In the presence of heavy infection and insufficient drug levels, an anti-malarial drug mutation typically arises and propagates. These spontaneous mutations are selected by different concentrations of anti-malarial drug that inhibit distinct genetic parasite types i.e. the drug concentrations are sufficient to reduce the susceptible parasite population, but cannot inhibit or cause less inhibition of the mutants (Peters, 1990). High drug pressure will eliminate susceptible ones and the acquired mutations allow the reproduction of the resistant parasite (Bloland, 2001).

Drug resistance to several anti-malarials is sometimes either due to changes in drug concentration in parasite vacuole by accumulation or efflux mechanisms (chloroquine, amodiaquine, quinine, halofantrine, mefloquine resistance) or due to point mutations in the target genes which leads to decreased affinity of the drug target (pyrimethamine, cycloguanil, sulphonamide, atovaquone, artemisinin resistance) (Foote and Cowman, 1994; Ward et al., 1995; Miotto et al., 2013; Ariey et al., 2014).
Administration of readily absorbed drugs with long elimination phases facilitates the spread of resistant malaria parasites. The remaining anti-malarial activity which remains present after the treatment serves as a “selective filter”, and prevents infection by sensitive parasites but enables infection resistant parasites. Drugs such as mefloquine, piperaquine and chloroquine, which remain in the blood for longer time, provide a selective filter long after their administration has stopped (WHO, 2010a). Uncontrolled use of drug, poor quality or fake drugs and cross-resistance also contribute to the emergence of drug resistance. The host immune response is also important factor to malaria infection which influences the spread of drug resistance and the extent to which drug resistance translates into clinical drug failure (Hastings, 2006).
Epidemiological studies have implicated low-transmission areas i.e. SE Asian region are the primary region where drug resistance arise (Roper et al., 2004). This is most likely attributable to the fact that areas of low transmission intensity and low immunity facilitate high parasitemia. In these areas, most of the malaria infections give symptoms and therefore proportionally many people receive treatment, provide more opportunities for selection of resistant strains. In high-transmission area, there is a less chance of emergence of drug resistance because in these areas asymptomatic infections are repeated throughout life. Also, in high transmission areas, malaria experienced populations slowly acquire partial immunity (Premunition), that in turn controls the infection. Some environmental factors and vector may possibly help in the propagation of resistant parasites (Wernsdorfer, 1994).

2.7 Mechanisms of resistance

Drugs act specifically by interfering with cellular or biochemical processes which are called 'targets'. The classical example, such as folate pathway or heme sequestration pathway, of a drug target can be an enzyme or gene which is inhibited by the drug. Parasites have evolved numerous ways to overcome the toxicity of drugs which leads to emergence of drug resistance. Mechanism of resistance involves mutations in target gene so that the drug does not bind or inhibit the target gene. Resistance also confers by changing in the copy number of the genes encoding to the drug’s parasite target or influx/efflux pumps that affect intra parasitic concentrations of the drug as described in Table 2. A single genetic event may be sufficient or multiple unlinked events may be required which will have additive or synergistic effects. Another mechanism of drug resistance involves increased production of target gene which can be accomplished through increased transcription and gene amplification, thus requires higher level of drugs to achieve the same level of inhibition. Decreasing drug accumulation can also contribute to drug resistance. The mechanism of resistance has been understood in some of commonly used anti-malarials i.e. chloroquine, the antifolates, and atovaquone, while still not fully resolved in case of Quinine, artemisinin and others.
Table 2- Target genes of different anti-malarial drugs confer resistance by mutational and copy number change

<table>
<thead>
<tr>
<th>Drug</th>
<th>Mutations or change in copy number</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinine</td>
<td>pfcr, pfmdr-1 and pfhe1</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>pfcr, pfmdr-1</td>
</tr>
<tr>
<td>Amodiaquine</td>
<td>pfcr, pfmdr-1</td>
</tr>
<tr>
<td>Mefloquine</td>
<td>pfmdr-1</td>
</tr>
<tr>
<td>Lumefantrine</td>
<td>pfcr, pfmdr-1</td>
</tr>
<tr>
<td>Atovaquone</td>
<td>Cyt b</td>
</tr>
<tr>
<td>SP</td>
<td>pfdhps, pfdhfr</td>
</tr>
<tr>
<td>Artemisinine</td>
<td>Pf kelch gene (k13)</td>
</tr>
</tbody>
</table>

2.7.1 Chloroquine

Chloroquine is most studied drug but its mechanism of action still remains to be elucidated. Most of the blood schizonicides drug target is localized in the parasite food vacuole (Geary et al., 1986b; Krogstad et al., 1987). Chloroquine (CQ) accumulates in the food vacuole of the parasite which may involve ions trapping following protonation, specific transport, and/or binding to a receptor (e.g. heme). The major action of chloroquine is to inhibit the formation of hemozoin (Hz), a toxic insoluble crystal also called malaria pigment, from the heme released by the digestion of hemoglobin (Hb). The free heme is toxic for parasite which can lyse membranes, lead to the generation of reactive oxygen intermediates, and leads to parasite death. Chloroquine also binds to heme to form complex entity which is highly toxic to the cell and disrupts membrane function. The actions of the toxic complex entity and free heme results in cell lysis and ultimately the auto-digestion of the parasite cell as seen in Figure 6.

Most of the drug targets are localized in the acidic food vacuole of the parasite which suggests that chloroquine resistance is due to decreased accumulation or extrusion of chloroquine in the food vacuole. It is believed that chloroquine resistance is due to increased
capacity for the parasite to expel chloroquine at a rate that does not allow chloroquine to reach levels required for inhibition of heme polymerization (Foley and Tilley, 1997).

In resistant parasite, this efflux occurs at a rate 40 to 50 fold faster among than in sensitive ones (Krogstad et al., 1987). In the laboratory, *P. falciparum* resistance to chloroquine and the efflux of chloroquine can be reversed with Ca+ channel blocker, such as verapamil and dilitazem (Krogstad et al., 1987; Martin et al., 1987) but clinical evidence is limited and the usefulness of this approach in humans has not been established (Bloland, 2001). Chloroquine resistance in *P. falciparum* has arisen spontaneously and probably a multigenic phenomenon

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**Figure 6-** Chloroquine metabolism in food vacuole of malaria parasite. Source- Tulane University webpage [http://www.tulane.edu/~wiser/protozoology/notes/drugs.html](http://www.tulane.edu/~wiser/protozoology/notes/drugs.html)
and is initially conferred by mutations in a gene encoding a transporter (pfcrtn) (Plowe, 2003) and mutations in a second transporter (pfmdrl) modulate the level of resistance in vitro. CQ resistance has been the identification of a 36 kb region on chromosome 7 of P. falciparum is associated with chloroquine resistance and specifically linked to the polymorphic gene pfcrtn (Fidock et al., 2000; Sharma, 2005). Lysine (K) to threonine (T) mutation at amino acid residue 76 (K76T) is the key mutation which is present in all documented clinical CQ failures and laboratory adapted field isolates of CQ-resistant strains, although other mutations are observed with different amino acid haplotypes (CVIETS, CVIDTS, CVMNTS, CVMETS & SVMNTS) corresponding to amino acid residues 72-76 and 220 respectively (Vinayak et al., 2003; Vathsala et al., 2004; Mittra et al., 2006). Another gene mdr-l, located on chromosome 5 of P. falciparum and coding for P-glycoprotein homologue-1 (Pgh-1) have generated interest in resistance to chloroquine and other anti-malarials. Different studies suggest that the point mutation of asparagines (N) to tyrosine (Y) at codon 86 (N86Y) is associated with chloroquine resistance (Duraisingh et al., 1997; Biswas et al., 2003). Several other pfmdrl polymorphisms i.e. Y184F, N1042D and D1246Y have also been associated with high level of chloroquine resistance.

2.7.2 Quinoline

Quinine, Mefloquine, Amodiaquine, Lumefantrine, other quinoline containing anti-malarials also appear to the affect of food vacuole of the parasite. However, exact mechanism is not clear whether these drugs bind heme or affect the formation of hemozoin because these drugs are weaker bases than chloroquine and may not exhibit the same degree of ion trapping as chloroquine within the food vacuole of the parasite.

Quinine is used to treat complicated and severe malaria cases for those patients who do not respond to CQ or SP treatment. The treatment failure cases are not very common in India but reduced efficacy of QN treatment is being noticed. Quinine resistance (QNR) may be a multigenic phenomenon involving several ion exchangers, P. falciparum Na+ /H+ exchanger (pfnhe-l) is mainly related to QNR (Sharma, 1997; Fidock et al., 2000; Babiker et al., 2001). This ion exchanger is also called ms 4760, seems to have variable number of DNNND repeats in a particular locus. However, molecular marker against QN resistance remains
elusive. The higher number of DNNND repeats were observed in the parasite lines which showed *In vitro* reduced susceptibility towards QN (Fidock et al., 2000; Babiker et al., 2001). Some studies revealed that the higher number of DNNND repeats were observed in high transmission areas isolates where level of CQ and SP resistance were also higher. Mefloquine resistance is associated with the copy number and polymorphism of *pfmdr*-1. However, the evidence on increased *pfmdr*-1 copy number for mefloquine resistance is still unknown. Some studies have suggested that a higher copy number confers mefloquine resistance (Price et al., 1999) but some other studies did not validate this fact from Brazil and Africa (Basco et al., 1995). Some studies have shown increased sensitivity to mefloquine with *pfmdr*-1 N86Y mutation (Price et al., 1999; Duraisingh et al., 2000) and suggests a possible inverse relationship between sensitivity to mefloquine and chloroquine because 86Y mutation of *pfmdr*-1 has shown increased sensitivity against mefloquine which is reverse in case CQ, while other mutations i.e. Y184F, N1042D and D1246Y were cause of resistance to mefloquine (Price et al., 1999; Reed et al., 2000).

### 2.7.3 Antifolate combination drug resistance

SP is one of the most important anti-malarial drugs used against falciparum malaria belongs to the antifolate combination drug class. However, at present, SP is used as a long acting partner drug in artemisinin combination therapy (ACT) to treat *P. falciparum* malaria patients. Folate metabolism is the target of several anti-malarials because reduced folates serve as a co-factor in a many reactions involved in the biosynthesis of amino acids and nucleotides. Due to its high rate of replication, the malaria parasite has a high demand for nucleotides as precursors for DNA synthesis so that very sensitive to antifolates. The malaria parasite synthesizes folates *de novo* and the inability of the parasite to utilize exogenous folates makes folate biosynthesis a good drug target. Folates participate as co-factors in many biosynthetic processes particularly in the synthesis of thymidylate (dTMP), which is desired for DNA synthesis. The two primary targets of anti-malarial drugs which target folate metabolism are DHPS and DHFR enzymes.

Folate is synthesized from 3 basic building blocks, GTP, p-aminobenzoic acid (pABA), and glutamate, in an enzymatic pathway involving 5 enzymes including DHPS and DHFR as important one. Dihydropteroate synthase (DHPS) is inhibited by sulpha-based drugs e.g.
sulfadoxine and dapsone. The sulfa drugs are structural analogs of pABA and compete for active site of DHPS and are converted into non-metabolizable adducts. This leads to a depletion of the folate pool which reduces the amount of thymidylate available for DNA synthesis as depicted in figure. Dihydrofolate reductase (DHFR) is a ubiquitous enzyme that participates in the recycling of folates by reducing dihydrofolate to tetrahydrofolate (Ferone, 1970). Then the tetrahydrofolate is oxidized back to dihydrofolate as it participates in biosynthetic reactions (e.g. thymidylate synthase). Pyrimethamine mimics dihydrofolate and compete for active site of DHFR as shown by Figure 7 and this inhibition will prevent the formation of thymidylate and lead to an arrest in DNA synthesis and subsequent parasite death. Pyrimethamine and proguanil are the commonly used anti-malarials. In case of \textit{P. falciparum}, sulfadoxine and pyrimethamine act synergistically to block DNA synthesis but their role in malaria is hampered by rapid emergence of resistance due to drug pressure (Plowe et al., 1997; Chulay et al., 1998). Specific point mutations in \textit{dhrs} and \textit{dhfr} gene of \textit{P. falciparum} lead to a lower affinity towards SP drug. Each mutation confers a stepwise reduction in susceptibility and resistance to the sulfadoxine and pyrimethamine, which are administered in synergistic combination, also results from sequential acquisition of mutations in the genes prior in \textit{dhfr} and then in \textit{dhrs} (Alifrangis et al., 2003). A single mutation can

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{f7.png}
\caption{Folate metabolism of malaria parasite. Source- Tulane University webpage http://www.tulane.edu/~wiser/protozoology/notes/drugs.html}
\end{figure}
lead to drug resistance but resistance tends to develop rapidly in the presence of high drug pressure. The use of combinations of drugs, having different mode of action will be evidence for slow the development of resistance because two independent mutations must occur for resistance to develop against both drugs. Combination of sulfadoxine and pyrimethamine “also known as” or “marketed by trade name Fansidar” is widely used for the treatment of uncomplicated falciparum malaria.

The development of resistance against SP emerges with a single point mutation in the parasite *dhfr* and *dhps* gene, which further augments with stepwise addition of mutations (Peterson et al., 1988; Triglia et al., 1997; Lozovsky et al., 2009). Resistance to pyrimethamine is primarily conferred by a point mutation from serine (S) to aspargine (N) at codon 108 is a key mutation for pyrimethamine resistance and augmented by mutations at codon alanine (A) to valine (V) at codon 16, aspargine (N) to isoleucine (I) at codon 51, cysteine (C) to arginine (R) at codon 59, and isoleucine (I) to leucine (L) at codon 164 of *Plasmodium falciparum dhfr* (*pf*dhfr) gene (Plowe et al., 1997; Peterson et al., 1988; Lozovsky et al., 2009). Similarly, point mutation at codon serine (S) to alanine (A) or phenylalanine (F) at codon 436 or alanine (A) to glycine (G) at codon 437 in *Plasmodium falciparum dhps* (*pf*dhps) gene may initiate the resistance and followed by mutations at codon lysine (K) to glutamic acid (E) at codon 540; alanine (A) to glycine (G) at codon 581; alanine (A) to serine (S) or threonine (T) at codon 613, which are considered for augmentation of sulfadoxine resistance (Triglia et al., 1997; Plowe et al., 1997; Gregson and Plowe, 2005).

### 2.7.4 Artemisinin

Artemisinin and its derivatives (e.g., artemether, artesunate, arteether and dihydro artemisinin) are highly potent, rapidly eliminated anti-malarial drugs which currently play important role in the control of multidrug resistant *P. falciparum* in many parts of South-East Asia. They clear parasitemia more rapidly than all other currently available anti-malarial drugs and are used for the treatment of uncomplicated and severe forms of malaria (White, 2004). The mechanism of action of act is incompletely understood, but previous studies revealed that artemisinin makes oxygen radicals in digestive vacuole where lots of oxygen and heme coexist which causes the depolarization of the mitochondrial membrane (Pandey et
al., 1999). Artemisinins have also been shown to inhibit \textit{pfATP6}, a SERCA-type enzyme (calcium transporter) and compete with thapsigargin for SERCA binding. Resistance to artemisinin is conferred by a single mutation in the calcium transporter (\textit{pfATP6}) associated with resistance to artemether, lending support to the idea that \textit{pfATP6} is the target for artemisinins (Eckstein-Ludwig et al., 2003; Krishna et al., 2010). Some more evidences of artemisinin resistance have recently been reported in Cambodia and Thailand (Noedl et al., 2010; Phyo et al., 2012).

Recently resistance to artemisinin derivatives has also been reported the reduced susceptibility of ring-stage parasites and slowly clearing infections (clearance half-life >5 hours) were strongly associated with single point mutations in the “propeller” region of the \textit{P. falciparum} kelch protein gene on chromosome 13 (kelch13) (Witkowski et al., 2013b; Ashley et al., 2014). Mutations that change the primary amino acid sequence of the propeller region of the kelch motif-containing gene, known as K13, and increased frequency of a dominant mutant K13-propeller allele in Cambodian provinces where resistance is prevalent correlates with the recent spread of resistance in western Cambodia. Strong correlations between the presence of a mutant K13-propeller allele, \textit{in vitro} parasite survival rates and \textit{in vivo} parasite clearance rates indicate that K13-propeller mutations are linked with artemisinin resistance (Witkowski et al., 2013a; Chotivanich et al., 2014). Artemisinin resistance emerged independently and spread in South East Asia so that \textit{K13}-propeller polymorphism acts as a useful molecular marker for large-scale surveillance of artemisinin resistance to prevent its global spread (Amaratunga et al., 2012; Hien et al., 2012; Phyo et al., 2012; Kyaw et al., 2013).

2.8 Origin of anti-malarial drug resistance

Drug-resistant strains have more often evolved in areas of low malaria transmission and low immunity that lead to high grade of parasitemia, such as Thailand and Cambodia, and then spread across to endemic areas like Africa, where they have contributed to worsening mortality. Most cases of resistance have emerged out in SE Asia region as an inherent tendency for the development of drug resistance through genetic mutation. Africa, areas with very high malaria transmission but less susceptible to the emergence of drug resistance
because infections in these areas are acquired repeatedly throughout life, resulting in partial immunity called premunition which in turn controls the infection below symptoms level.

In 1955, WHO launched the most ambitious Global Malaria Eradication Programme (GMEP) in which mass administration of chloroquine was started as a preventive measure in many areas, particularly in SE Asia and South America, and it was also administered to even those who did not have malaria. As a result of this widespread use, in late 1950s resistance to CQ in *Plasmodium falciparum* was first observed in SE Asia (Thai-Cambodian border) and simultaneously in South America (Colombia) and now found in all malaria endemic regions of world (Young et al., 1961; Spencer, 1985; Wernsdorfer et al., 1991). Chloroquine was also given to migrant worker as a prophylaxis treatment to prevent malaria (Packard, 2014). In 1969, chloroquine resistance was spread to Myanmar and Bangladesh from Western Cambodia through migrant worker and then reached to neighboring Karbi-Anglong district of Assam, India by 1973 (Sehgal et al., 1973; Farooq et al., 2004; Shah et al., 2011; Packard, 2014). In 1978, CQ resistance was first appeared in the eastern part of Africa and then spread to the central and southern parts of Africa in 1983 (Peters 1987; Wernsdorfer et al., 1991; Pickard et al., 2002). With chloroquine losing its efficacy, sulphadoxine-pyrimethamine combination was introduced in the SE Asia region, as a drug of choice for the treatment of chloroquine resistant malaria. SP resistance was first reported also from the Thai-Cambodian border in *P. falciparum* in the year of its introduction in 1967, and it reached India by 1979 (Sehgal et al., 1973; Farooq et al., 2004; WHO, 2010a; Shah et al., 2011) and now it is frequently seen in South America and Africa. The first case of quinine resistance was described from Thai-Cambodian border in mid 1960s (Pickard et al., 2002).

During the Viet Nam War, mefloquine was developed by American Army for their soldiers, but it came to use after the war ended. Mefloquine was introduced in Thailand in 1977, and mefloquine resistance was first appeared in late 1980s also near the Thai-Cambodian border (Shanks, 1994; Wongsrichanalai et al., 2001) and cross-resistances to quinine and halofantrine is also a cause of mefloquine resistance (Bloland, 2001; WHO, 2014b). In India, mefloquine resistance in *P. falciparum* was reported from Surat district in Gujarat state (Sharma, 1996).
By 1991, Viet Nam started production of artemisinin and started using it, mainly as monotherapy. In late 2000s, parasite resistance to artemisinins has been detected and then WHO banned use of oral artemisinin as monotherapy which is considered to be a major contributing factor to the development of artemisinin resistance. In 2001, WHO recommended ACTs as the first-line treatment against \textit{P. falciparum} malaria, combining a fast acting artemisinin compound with another slower acting anti-malarial such as SP, mefloquine, amodiaquine or lumefantrine. The use of ACTs has been going up all over the world, and it was seen that after 2005 there was a substantial decline in outbreak of this disease (WHO, 2012).

However, some recent studies revealed that parasites which are resistant to artemisinin and its derivatives have recently detected in five countries i.e. Cambodia, Lao People’s Democratic Republic, Thailand, Viet Nam and Myanmar, which is a major threat for malaria control strategies, treatment and elimination efforts (Enserink, 2010; Dondrop et al., 2011; Ashley, et al., 2014; WHO, 2014c).

### 2.9 Determination of drug resistance

The rapid progression of anti-malarial drug resistance has emphasized the need of continuous monitoring of drugs to make effective malaria treatment policies. High priority should be given to new methods that facilitate the accurate assessment of the drug response. The available monitoring procedures include the therapeutic efficacy test (\textit{in vivo} tests), in which repeated assessment of parasitological and clinical outcomes of a treatment examined during a fixed period of follow-up. \textit{In vitro} studies and molecular markers are other available methods in which parasite susceptibility to drugs in culture and gene mutations or gene amplification linked with parasite resistance are measured respectively (White, 2002).

#### 2.9.1 \textit{In vivo} tests/ therapeutic efficacy test:

Soon after the first well documented reports of CQ resistance in Thailand and South America, in 1965, WHO established the most traditional approach, that is assessment of therapeutic (\textit{in vivo}) response of a drug which was defined in terms of parasite clearance. The therapeutic efficacy test remains the gold standard for monitoring anti-malarial drug efficacy.
and also provides national malaria control programs and drug policy makers a straightforward indicator of the efficacy of an anti-malarial drugs to suggest whether a drug is still efficient or not in a given population at risk.

*In vivo* tests are based on the examination of parasite response in the malaria patients to a fixed dose of a drug within the limits of tolerability and estimation of treatment failure rate and confirmation of drug resistance can only be done by this method. In starting, the evaluation of *In vivo* drug response of *P. falciparum* to anti-malarials requires longer period of follow up i.e. 28days including seclusion of patients to prevent the reinfection. Later in 2003, WHO recommended a protocol with some modifications, having shorter period of follow up of 7-14 days (“WHO standard test”) under the hypothesis that reappearance of parasites within 14 days of treatment is due to recrudescence than reinfection (WHO, 2003). Traditionally response to treatment was categorized purely based on parasitological response as sensitive (S) and three other degree of resistance RI, RII and RIII (WHO, 1973; Bruce – Chwatt, 1986). In high transmission areas re-infection is difficult to exclude, so the WHO introduced a modified protocol based on clinical outcomes (adequate clinical response, early treatment failure and late treatment failure) where parasitemia in the absence of clinical signs or symptoms is common (WHO, 1996). These tests have to be carried out with standard therapeutic doses of drugs within the limits of tolerability thus they do not permit a quantitative assessment of the drug sensitivity of individual parasite populations beyond the question of treatment failure or success. These studies are performed in a controlled environment, in which drug administration is supervised and the results are validated properly. Therapeutic efficacy tests also have some drawbacks that outcome of such a study is influenced by a combination of a human factor (immunity), a parasite factor (drug resistance), variation of drug absorption and misclassification of re-infection and recrudescence.

### 2.9.2 *In vitro* drug susceptibility test:

The problem associated with the assessment of anti-malarial drug resistance in *in vivo* studies has led to the introduction of *in vitro* tests which give more accurate result of drug sensitivity under controlled experimental conditions. These test results have complete exclusion of host-
related factors, such as drug failure or host immunity, so results from in vitro studies provide a more objective insight about drug sensitivity than do in vivo tests. The commonly used methods for the anti-malarial in vitro testing are the in vitro WHO Mark III test, the radio-isotopic test and the enzyme linked immunosorbent assay directed against Plasmodium lactate dehydrogenase (pLDH) or histidine-rich protein II (HRP2) and more recently the SYBER Green test (Noedl et al., 2003). These tests include the estimation of the parasite metabolic process in short-term or long-term cultivation of P. falciparum parasite in vitro with a different drug concentration of anti-malarials. The data obtained from in vitro studies have to be validated also with in vivo tests and pharmacological tests to find out individual sensitivity levels for the drug tested. These studies provide quantitative results and multiple tests can be performed with a single parasite isolate against several drugs and drug combinations simultaneously (Bloland, 2001). Experimental drugs response can also be assessed by these methods easily. In vitro tests have proven useful in epidemiological monitoring of different anti-malarials to provide temporal and spatial changes of drug sensitivity as an early warning and guidance for therapeutic studies and also for validation of molecular markers of drug resistance. In vitro tests have some limitation including requirements of expensive equipment and supplies along with trained man power are essential. Some other drawbacks of this study are presence of mixed parasite populations having different drug sensitivity and correlation with efficacy tests not fully established. Drug sensitivity of P. vivax is considerably more difficult to assess by in vivo tests as compared with P. falciparum because it produce relapse from hypnozoites (White, 2002). Unfortunately, establishment of P. vivax continuous culture have effectively failed so far but short-term culture of P. vivax was reported many years ago (Bass and Johns, 1912; Chotivanich et al., 2001).

2.9.3 Molecular marker:

Molecular markers for drug resistance are a great tool for epidemiological monitoring of drug resistance for a whole country as well as for prediction of therapeutic results of a single individual. These tests are basically based on PCR analysis in which small amount of parasite DNA taken from finger-prick dried blood spots on filter paper, where one sample can be used for multiple tests and also molecular targets of several drugs to be characterized. Molecular
markers are based on detection of gene mutations or amplification that modifies drug target gene or drug transporter functions which are associated with resistance to a number of antimalarials. Molecular markers of resistance have been established only for some drugs i.e. chloroquine (chloroquine resistance transporter gene, \textit{crt} and multidrug-resistance gene-1, \textit{mdr-1}), pyrimethamine (dihydrofolate reductase gene, \textit{dhfr}), sulfadoxine (dihydropteroate synthase gene, \textit{dhps}) and artemisinin (ATPase6, \textit{mdr-1} and \textit{K13}) (Wernsdorfer and Noedl, 2003; Sharma, 2005). An increased copy number and polymorphisms in \textit{P. falciparum} multidrug-resistance gene 1 (\textit{Pfmdr-1}) have been recognized as a marker of mefloquine and quinine resistance and mutations in \textit{Pfmdr-1} have also been associated with the sensitivity to artemisinin but some field studies have provided contradictory results (Duraisingh et al., 2000; Price et al., 2004). Specific point mutation at codon 268 of \textit{cytochrome b} gene has been associated with treatment failures in atovaquone–proguanil (Gil et al., 2003; Wichmann et al., 2004). Merozoite surface proteins 1 and 2 (\textit{msp-1} and \textit{msp-2}) and glutamate rich protein (\textit{glurp}) are the genetic markers of recrudescence and re-infection (Snounou and Beck, 1998). These tests can provide early warning of impending resistance and are also helpful for monitoring susceptibility changes over a time period against a drug that has been withdrawn from the field (Mita et al., 2003). The usefulness of molecular markers has importantly marked with the ever increasing use of combination therapy. These methods can be implemented at a bigger scale to screen the larger parasite populations from the field, as they can study many isolates within a short time and collection, storage and transport of samples for molecular analysis are far easier than \textit{in vitro} and therapeutic efficacy test. Although monitoring of molecular markers of resistance is simple than \textit{in vitro} sensitivity methods, but the molecular methods also have their own drawbacks. An important issue is that the detected mutations and its relation with therapeutic efficacy test are not fully established. Therefore, attempts were made to identify the molecular markers to detect the drug resistance status of the parasite by molecular tools. Molecular markers against different drugs are listed in Table 3.
<table>
<thead>
<tr>
<th>Anti-malarial drug</th>
<th>Introduction date</th>
<th>First reported resistance</th>
<th>Molecular marker</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinine</td>
<td>1632</td>
<td>1910</td>
<td><em>pfhne</em>: microsatellite ms4670</td>
<td>Peters, 1987; Ferdig et al., 2004; Henry et al., 2009; Okombo et al., 2010</td>
</tr>
</tbody>
</table>
| Chloroquine             | 1945              | 1957                      | *pfcret*: C72S, M74I, N75E/D, K76T, H97L, A220S  
*pfmdr-1*: N86Y, Y184F, S1034C, N1042D, D1246Y  
*pfmdr-2*: N86Y, Y184F, S1034C, N1042D, D1246Y  
*pfmdr-3*: N86Y, Y184F, S1034C, N1042D, D1246Y | Wernsdorfer and Payne, 1991; Djimde et al., 2001; Bray et al., 2005; Duraisingh and Cowman, 2005 |
| Mefloquine              | 1977              | 1982                      | Deamplification of *pfmdr-1* copy                   | Wongsrichanalai et al., 2001; Imwong et al., 2010                      |
| Halofantrine            | 1988              | 1993                      | Changes in *pfmdr-1* copy number                   | Wernsdorfer et al., 1994 ;Bouchaud et al., 2009; Van Tyne et al., 2011 |
| Artemisinin             | 1971              | 1980                      | Amplification of *pfmdr-1* copy numbers and mutation of *pfATPase6*.  
Recently, mutation in K13-Propeller Domain has been confirmed. | Rosenthal et al., 1991; El-Ali et al., 2006; WHO, 2006; Takala-Harrison et al., 2014 |
| Artesunate              | 1975              | 2008                      | NA                                                    | Noedl et al., 2008                                                        |
| Artesunate + Mefloquine | 2000              | 2009                      | Deamplification of *pfmdr-1* copy                   | Rosenthal et al., 1991; Dondrop et al., 2009; Maude et al., 2009          |
2.10 Anti-malarial drug resistance status

With the availability of various anti-malarial drugs, the chloroquine was the only choice for many decades because of its safety, efficacy and affordability. However, parasite resistance to this drug was initially documented in Thailand and Cambodia border in 1957 and then on the border of Colombia and Venezuela in 1959. After that, CQ resistance has widespread towards the tropical world. In African countries, chloroquine resistance was first appeared in Tanzania and has spread and intensified across the continent in the late 1970s. Then CQ resistance was detected in East Africa and by the mid-1980s had become a major problem in across the continent (Wernsdorfer and Payne, 1991). At present, chloroquine remains effective only in some areas of Central America, but clinical failure rates of chloroquine reached up to 70–80% in several countries i.e. Asia, Africa and also in the America (Londoño et al., 2009). Chloroquine resistance in *P. vivax* is rare which was first reported in 1989 in Papua New Guinea and then cases have been reported from Indonesia, Myanmar, India, Borneo, Vietnam, Peru, Turkey and Ethiopia (Baird et al., 2004). However, significant proportion of chloroquine resistance is reported largely from Indonesia, East Timor, Papua New Guinea and some other parts of Oceania, while northeastern coast of Indonesian Papua reported chloroquine resistant *P. vivax* with highest prevalence (84%). According to WHO, *P. vivax* remains sensitive to chloroquine drug in most part of the SE Asia, the Indian subcontinent, Korea, the Middle East, Northeast Africa and South and Central America (Teka et al., 2008; WHO, 2014a). In India, chloroquine resistance was first reported from Karbi-Anglong district of Assam in 1973. Thereafter, chloroquine resistance has spread towards the south and west parts and covered almost the entire country (Clyde, 1987). Currently, chloroquine resistant *P. falciparum* malaria has been observed with increasing frequency in northeast and southeastern regions of India with high morbidity and mortality (Sharma, 2012).

Amodiaquine is generally more effective than chloroquine in areas of persistent CQ resistance. Due to their efficacy, amodiaquine was used in in combination with artesunate as the first-line treatment in several countries. Recent studies reported that in Tanzania high
resistant parasite to amodiaquine are prevalent, which may possibly affect the use of artesunate plus amodiaquine in Africa (Sa et al., 2009). Resistance level of quinine is low and still effective against *Plasmodium* species but quinine resistance has also been reported from parts of South East Asia and South America (Bloland, 2001; Wongsrichanalai et al., 2002). Mefloquine was introduced in South East Asian region in 1977 and it became ineffective by 1982. Increased level of mefloquine resistance is also due to cross-resistance to halofantrine and quinine (Bloland, 2001). Presently, mefloquine resistance is prevalent in Myanmar, Thailand, Cambodia, and Vietnam where artesunate-mefloquine is still used as first line treatment (Satimai et al., 2012). In Africa, mefloquine resistance is rare but low level of mefloquine resistance has been found in Amazon region. Resistance to mefloquine continues to bee a serious concern in the Great Mekong sub-region, in particular in Thailand and Cambodia, where artesunate plus mefloquine is still used as first-line treatment (Satimai et al., 2012). When chloroquine was losing its efficacy, SP was introduced as a first-line treatment against chloroquine-resistant *P. falciparum* parasite in the SE Asia region, only to be vanished to resistance. However, resistance to sulfadoxine-pyrimethamine was reported in *P. falciparum* with in the year of introduction in 1967, and it reached India by 1979. Recently high levels of resistance to sulfadoxine-pyrimethamine are frequently seen in many parts of South East Asia, eastern and southern Africa, and the Amazon region (Farooq et al., 2004; WHO, 2010a; Shah et al., 2011). In case of *P. vivax*, there is widespread resistance of pyrimethamine while sulfa drugs are less active against *P. vivax* than *P. falciparum* because *P. vivax* has shown native resistance to sulfadoxine. Therefore, the synergy of sulfa drugs and pyrimethamine is not effective against *P. vivax* but SP resistant parasite has been reported in many areas (Imwong et al., 2001; Korsinczky et al., 2004). Some recent study found the failure of primaquine therapy in *P. vivax* malaria, from different regions including some parts of India which may be due to recrudescence of chloroquine-resistant strains or due to reinfection. In India, Resistance in *P. falciparum* to SP combination was first reported in 1987 (Choudhury et al., 1987) and then prevalence of SP resistance has been reported from *P. falciparum* prevalent areas like northeast states and Orissa (Ahmed et al., 2004; Shah et al., 2011; Mishra et al., 2014). Since 2005, ACT therapy (AS+SP) was used for the treatment of SP resistant *P. falciparum* parasite in India and later in 2010, this treatment recommended as first line treatment throughout India (National Drug Policy, 2010; Shah et
al., 2011). In north east India, since 2013, there is increased prevalence resistance in *P. falciparum* against this partner drug SP which led to introduction of artemether-lumefantrine as anti-malarial therapy in this region (National Drug Policy, 2013; Mishra et al., 2014).

Rapid increase in drug resistance against all conventional anti-malarials, make sure that at present artemisinin and its derivatives have been the key line of defense against drug resistant malaria in many parts of South-East Asia. In 2001, WHO official recommended that artemisinin-based combination therapies (ACTs) as the first-line treatment of *P. falciparum* malaria and then there was a substantial decline in outbreak of this disease after 2005 (WHO, 2012). In order to get maximum effectiveness of artemisinin and its derivatives and also protect them from the development of resistance or to delay resistance, WHO has recommended that these drugs can be combined with other drugs having longer half lives and have different mechanisms of action. At present, five combinations are currently recommended by WHO: artemether-lumefantrine, artesunate-amodiaquine, artesunate-mefloquine, artesunate-SP and dihydroartemisinin-piperaquine (WHO, 2010c). However, the drug resistant parasite against artemisinin and its derivatives have recently emerged in various parts of South East Asia, which threaten, all prior success of malaria control strategies (Enserink et al., 2010; Dondorp et al., 2011).