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A Comprehensive Review of Malaria with an Emphasis on Plasmodium Resistance

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A COMPRENHENSIVE REVIEW OF MALARIA WITH AN EMPHASIS ON *PLASMODIUM* RESISTANCE

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ABSTRACT

Malaria is a disease that is caused by the *Plasmodium* genus. It is endemic in tropical areas. There are multiple drugs used for prophylaxis and treatment. However, the parasites have developed resistance towards most antimalarial pharmaceuticals. The pharmaceutical industry is generating new antimalarial pharmaceuticals, but the rate of new *Plasmodium* resistance is much faster.

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LIST OF ABBREVIATIONS

CDC	Centers of Disease Control and Prevention
WHO	World Health Organization
ACT	Artemisinin Combined Treatment

Introduction

Malaria is a disease caused by the *Plasmodium* genus that is transmitted between humans by *Anopheles* mosquitoes. *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale, Plasmodium knowlesi,* and *Plasmodium malariae* are the species of *Plasmodium* that invade humans. *P. falciparum* and *P. vivax* are the most common species that cause malaria in humans. *P. falciparum* is the most dangerous because of the multi-drug resistance on this strain of the disease.[1] Malaria is both curable and preventable with medication therapy; however, a vaccine is not available.

According to the World Health Organization, in 2012, there were approximately 207 million cases of malaria resulting in 627,000 deaths.[2] The overwhelming majority, 90%, of these cases happen in Africa.[1] Most of the deaths occur in children. However, the rate of deaths in children has been reduced by 54% since 2000.[2] The distribution of worldwide confirmed cases are shown in *figure 1*. Countries with the most confirmed cases are in sub-Saharan Africa and India. In the United States, most of the cases of malaria are due to international travelers that acquired the disease while visiting an endemic country.[2]

Figure 1

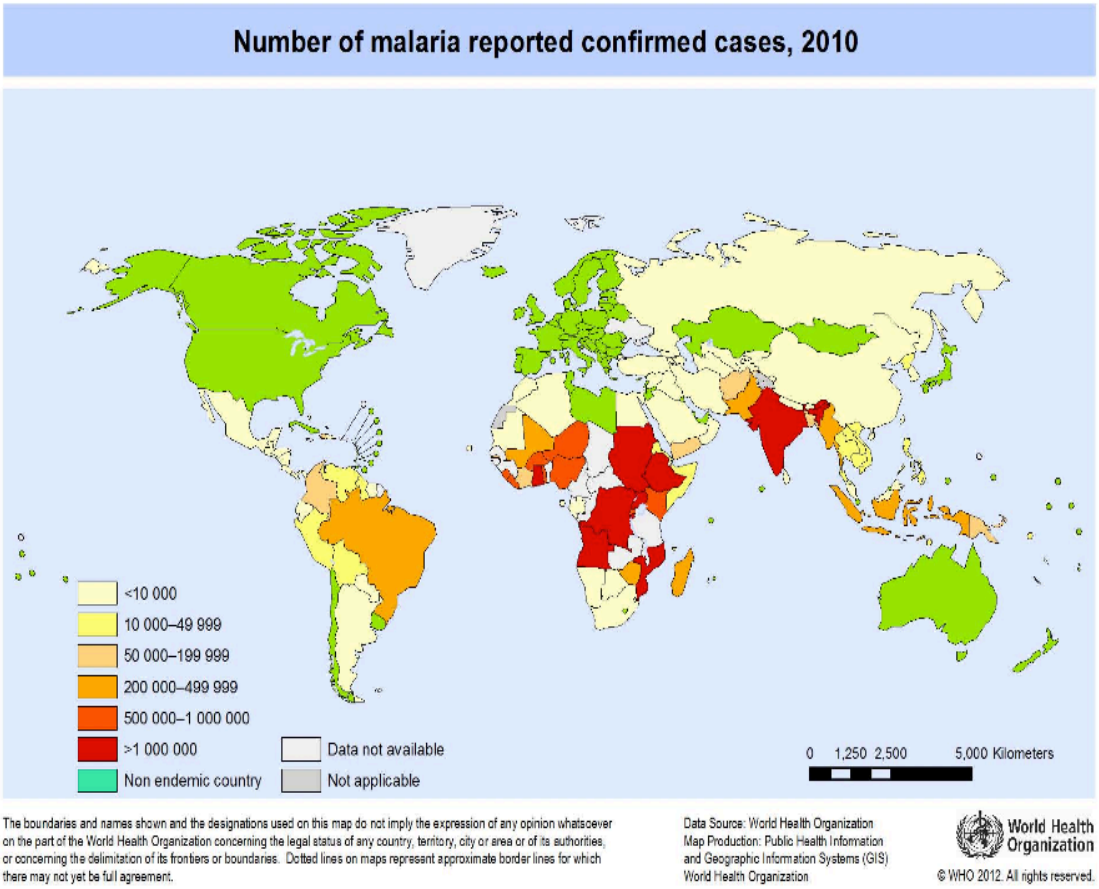

Figure 1. This is a map from the World Health Organization showing the number of confirmed cases of malaria in 2010.

The major problem with antimalarials is the resistance that *Plasmodium* is developing towards these medications. The CDC defines antimalarial drug resistance as "the ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject."[3] The *Plasmodium* genus has a complex genome that allows the parasite to switch between the two hosts. Resistance occurs through multiple spontaneous mutations that reduce the sensitivity of the strains to the antimalarial medications. When large populations of *Plasmodium* are

exposed to the drug, the rate of resistance increases greatly, because the parasites' sensitivity to the medications are removed. However, the resistant strains will survive and multiply. Most of the mutations that cause resistance affect the erythrocyte stage of the *Plasmodium,* which affect the metabolism and action of the medication.[4] This review will be focusing on the antimalarial pharmaceuticals available in the United States.

Chapter I: Background

Malaria has existed for over 4,000 years. The first description of the disease was in ancient Chinese medical writings, *Nei Ching* (The Canon of Medicine), around 2700 BCE. By the fourth century BCE, the Greeks had documented the disease. Malaria had been the cause of a great decrease in population of the Greek city-states. Hippocrates documented the symptoms. The symptoms of malarial fever were documented in the *Susruta,* a Sanskrit medical treatise. The treatise first connected the bite of an insect to the cause of malarial fever.[5]

The Chinese discovered the first medication used for the treatment of malaria during the second century BCE. *52 Remedies*, a medical treatise, documented of the use of the Qinghao plant (*Artemisia annua*) against malaria. However, it was not until 340 CE when the antifever qualities of the plant were described in medical text. Artemisinin, the active ingredient of the Qinghao plant, was not isolated until 1971 by Chinese scientists. Artemisinin drug products are used today as antimalarial drugs in areas where there is resistance to chloroquine.[5]

The Spanish Jesuit missionaries documented the use of another medication in the early seventeenth century. The medication was found in the bark of a tree in Peru.

The bark had been recognized when it cured the Countess of Chinchon, the wife of the Viceroy of Peru, who had contracted the malarial fever. The tree was named Cinchona after the countess, and the bark was called *Peruvian bark*. Quinine, an antimalarial used today, is derived from *Peruvian bark*.[5]

On November 6th, 1880, in Constantine, Algeria, Charles Louis Alphonse Laveran, a French army surgeon, was treating a patient with malaria. When the French army surgeon examined the patient's blood, he noticed a parasite in the blood. He believed there was only one parasite that caused the disease and he named it *Oscillaria malariae.*[3] He was also able to discover part of the life cycle of malaria, because he observed the exflagellation of the male gametocyte in the blood. The motile gametocyte led him to believe the parasite was a protozoan. By not finding the parasite in the air, water, or soil of the marshland, Laveran was able to deduct that the parasite must live inside the mosquito.[6]

Six years after the parasite was discovered in the blood of a patient with malaria, Camillo Golgi determined there were multiple forms of the disease. He came to this conclusion by observing the fever trend of patients with malaria. He discovered that one form was characterized by having a fever every second day (tertian periodicity) and another form had a fever every third day (quartan periodicity). He was able to connect the fever with rupture of the red blood cell due to the release of mature merozoites that had accumulated in the cell.[5]

In 1890, Giovanni Batista Grassi and Raimondo Filetti named *Plasmodium vivax* and *Plasmodium malariae*, which were the first species of *Plasmodium* to be named. In 1897, William H. Welch renamed *Oscillaria malariae*, *Plasmodium*

falciparum, which is the cause of tertian periodicity fever. In 1922, John William Watson Stephens named and described *Plasmodium ovale.* In 1931, Robert Knowles and Biraj Mohan Das Gupta discovered *Plasmodium knowlesi* in a long-tailed macaque (a type of monkey). The first human case of malaria caused by *P. knowlesi* was documented in 1965.[5]

Ronald Ross was the first to prove *Plasmodia* were transferred from humans to mosquitoes in 1897. He observed the infected mosquitoes bit birds, which contracted malaria afterwards. In 1899, Grassi, Amico Bignami, and Guispeppe Bastianelli demonstrated that the sporogonic (sexual) cycle of the *Plasmodium* genus occurs in the *Anopheles* mosquito. They collected mosquitoes and let them bite malaria infected humans. Then, the investigators allowed the mosquitoes to bite healthy volunteers, whom consequently developed malaria. This led to the discovery of the transmission cycle of Malaria in humans.[5]

Chapter II: Life cycle of Malaria

The life cycle of malaria has two stages, a sexual stage and an asexual stage. The sexual stage occurs in an invertebrate definitive host. In malaria, the definitive host is a female *Anopheles* mosquito. The asexual stage occurs in an intermediate vertebrate host, which is the human.[7] *Figure 2* shows the life cycle of Malaria.

Figure 2

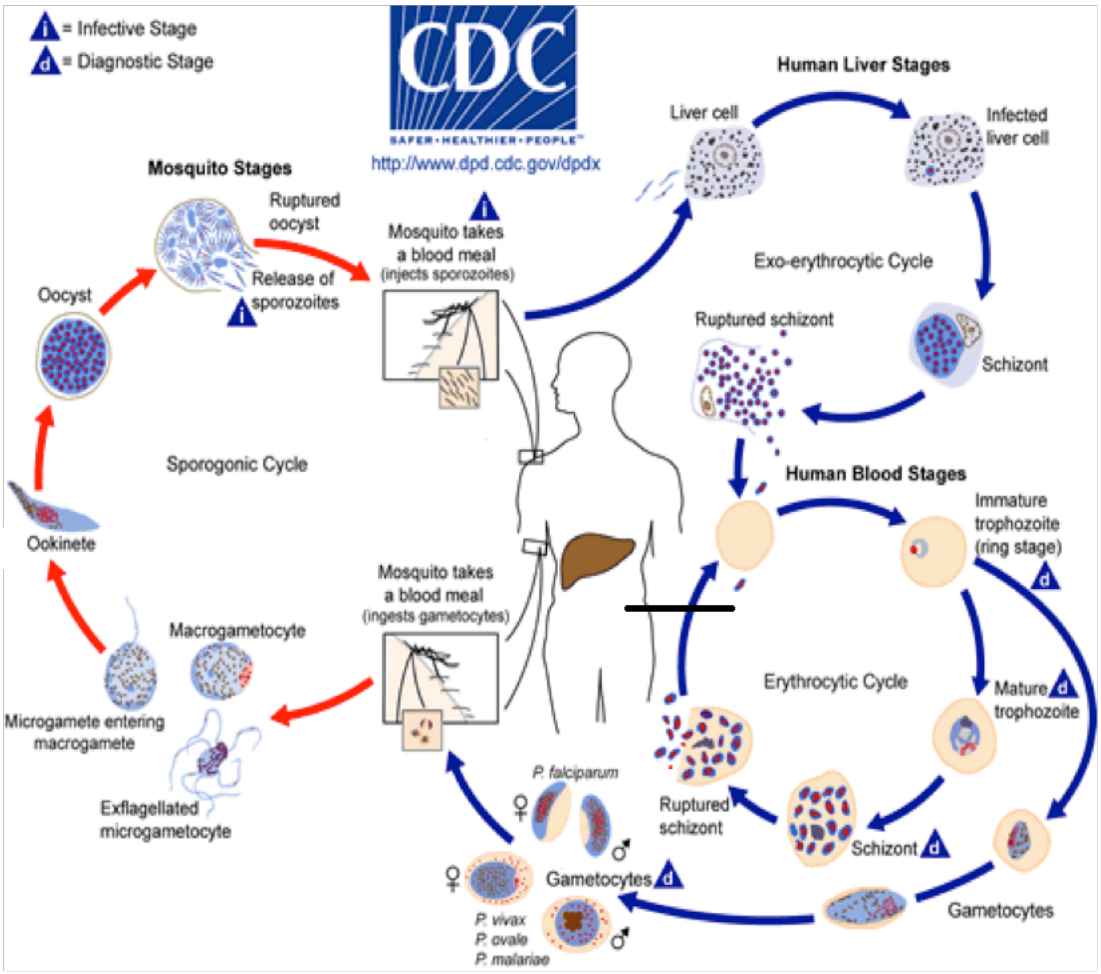

Figure 2. This figure shows the life cycle of malaria in the *Anopheles* mosquito and the human host. The Life Cycle of Malaria- Biology [Internet]. Centers for Disease Control and Prevention. Available from: http://www.cdc.gov/malaria/about/biology/

The sexual stage, also known as the sporogonic cycle, begins when a female *Anopheles* mosquito feeds on a human infected with a *Plasmodium*. The red blood cells the mosquito ingests contain male and female *Plasmodium* gametocytes. In the mosquito, the male gametocytes, or microgametocyte, undergo exflagellation to form mature male gametes. At the same time the female gametocytes, or macrogametocytes, come together in the human red blood cell. The female

gametocytes shed the red blood cell becoming female gametes. The male gametes penetrate the female gametes forming a zygote.[3,8]

The zygote elongates into an ookinete, which infiltrates the stomach of the mosquito and attaches to the outer lining of the stomach. As the ookinetic enlarges it forms into an oocyst. The oocyst rapidly divides into sporoblasts, which do not have a defined nucleus. Once the sporoblasts develop a defined nucleus and break away from the stomach lining, they become sporozoites. The sporozoites migrate and invade the salivary gland of the mosquito. This normally takes 10 to 18 days after the intake of gametocytes. This is the end of the sexual stage of the life cycle of *Plasmodium*.[3,8]

The asexual stage begins when the sporozoites are transferred to a human host when the infected mosquito feeds. The asexual stage can be further divided into the exo-erythrocyte stage and the erythrocyte stage.[3]

The exo-erythrocyte stage occurs first, because the sporozoites travel to the liver of the human host and invade the hepatocytes.[3] In the hepatocytes, the sporozoites mature into schizonts. After five to sixteen days, the schizonts rupture, they release merozoites, the haploid form of *Plasmodium*.[8] *Plasmodium vivax* and *Plasmodium ovale* can remain in the hepatocyte in a dormant stage. The merozoites in the dormant phase are called hypnozoites.[8] The merozoites exit the hepatocytes and enter the blood stream. Once the merozoites enter the blood stream, they penetrate the red blood cells of the human host. This begins the erythrocyte stage.[9]

In the erythrocyte stage, the merozoites join together to form ringed trophozoites in the red blood cells.[10] The parasite consumes the hemoglobin through

its food vacuole. The hemoglobin is metabolized into heme, which is toxic to the parasite. So the parasite uses polymerase to detoxify heme into crystals of hemozoin pigment.[11] The trophozoites mature into schizonts or into gametocytes.[10] The schizont phase will rupture into merozoites and repeat the erythrocyte stage. The gametocytes are crescent-shaped and mature very slowly (around 10 days).[10] The gametocytes are eventually ingested by the *Anopheles* mosquitoes starting the sexual stage.[8]

Chapter III: The Disease

Malaria can be categorized in two categories: uncomplicated or complicated (severe). If malaria is promptly diagnosed and treated, it is curable. The symptoms of malaria are associated with the erythrocyte stage, because of the waste and toxins caused by the destruction of the red blood cell by *Plasmodium*.[12]

Malaria is usually diagnosed by the presence of *Plasmodium* in the patient's blood. Mild anemia, thrombocytopenia, increased bilirubin, and increased aminotransferases can also be helpful laboratory findings to diagnosis malaria.[12]

Uncomplicated Malaria

Classical uncomplicated malaria has three stages: a cold stage, a hot stage, and a sweating stage. The cycle lasts between 6-10 hours. The cold stage consists of shivering. A patient in the hot stage suffers from fever, headaches, vomiting, and seizures (frequently in young children). The sweating stage consists of sweats and tiredness.[12]

Tertian and quartan periodicities are associated classical attacks. In tertian attacks the symptomatic stages occur every second day. These attacks are caused by

P. falciparum, P. vivax, and *P. ovale*. In quartan attacks, the symptomatic stages occur every third day. *P. malariae* is the cause of quartan periodicity.[12]

Uncomplicated malaria can be misdiagnosed as influenza or the common cold. This occurs in countries where malaria is not common; therefore, the patient is not expected to have malaria. In endemic areas, malaria symptoms are recognized.[12]

Severe Malaria

A patient has severe malaria when he or she suffers from organ failure or abnormalities in the blood or metabolism. Severe malaria is associated with cerebral malaria, severe anemia, hemoglobinuria, acute respiratory distress syndrome (ARDS), abnormal blood coagulation, low blood pressure, acute renal failure, hyperparasitemia (more than 5% of erythrocytes are infected with parasites), metabolic acidosis, and hypoglycemia. ARDS inhibits oxygen exchange through inflammation. Severe malaria requires urgent and aggressive treatment.[12]

Incubation period

After an infected *Anopheles* mosquito, there is an incubation period that lasts from seven to thirty days. *P. falciparum* infections are associated with shorter incubation periods, while *P. malariae* is associated with longer incubation periods. During the incubation period, the patient will not have any symptoms. The incubation period can be pro-longed if the patient had taken antimalarial pharmaceuticals for prophylaxis. *P. vivax* and *P. ovale* infections both can have delayed onset of symptoms, since theses parasites can go into a dormant phase of the liver.[12]

Symptoms and Complications

The majority of complications and symptoms of malaria in the human host are associated with the erythrocyte stage of *Plasmodium*.[8] The *Plasmodium* destroys the inside of the erythrocyte, which causes waste and toxins to build up in the infected red blood cell. When the merozoites cause the red blood cell to rupture, the toxins and wastes products enter the blood stream. Macrophages and other cytokine-producing cells are attracted to the waste products, such as hemozoin, and toxins, such as glucose phosphate isomerase. Resulting symptoms are fever and rigors.[12] Moderate to severe shaking, chills, high fever, and profuse sweating occur in cycles. Headache, vomiting, and diarrhea may also occur.[13]

Malaria causes severe complications, and if left untreated usually result in death. These complications include cerebral malaria, breathing problems, organ failure, severe anemia, and low blood sugar. Cerebral malaria occurs when the infected erythrocytes occlude the small vessels that lead to the brain. This causes brain swelling or damage, which leads to coma. Breathing problems occur because of the accumulation of fluid in the lungs. Organ failure occurs in kidneys or liver malaria may cause spleen rupture. Severe anemia happens because the parasite destroys the red blood cells. Malaria and certain malarial drugs can cause low blood sugar.[14] *P. falciparum* infections can cause cerebral malaria. Red blood cells with mature trophozoites stick to the vascular walls of small blood veins. This causes a

blockage of blood flow. Cerebral malaria occurs when the blockage is in a vein of the brain. This complication has about a 20% mortality rate.[12, 15]

Sickle cell trait

In people possessing the sickle cell trait, a protective advantage against malaria is apparent. The sickle cell trait is one normal hemoglobin gene and one sickle hemoglobin gene. Sickle cell gene affects the beta chain of the hemoglobin. It is a single amino acid change of glutamate to valine at the 6th position. The erythrocytes affected by this mutation have a shorter life. Heterozygotes for the sickle genes show lower numbers of erythrocytes infected with *Plasmodium* and decrease incidence of severe complications of malaria, such as cerebral malaria and severe anemia. However, homozygotes do not have a protective advantage and are highly susceptible to the lethal effects of malaria. The frequencies of the sickle cell trait are high in malaria-endemic areas.[16]

Chapter IV: Blood Schizonticides

Chloroquine

General Drug Information

Chloroquine phosphate, an antimalarial drug, 4-aminoquinoline, was discovered in 1934. It is the most commonly used medication to treat acute, uncomplicated cases of malaria. Chloroquine can be administered to treat *Plasmodium ovale*, *Plasmodium vivax,* and *Plasmodium falciparum.* However, many strains of *P. falciparum* and *P. vivax* have developed resistance to chloroquine because of the over-use of chloroquine. This unfortunate consequence has resulted in chloroquine being less effective in countries where resistance has been demonstrated.[5]

Chloroquine is highly effective during blood stages of the life cycle of malaria; however, it is not effective against the exo-erythrocyte stage of the parasite nor when relapse occurs in certain cases of *P. vivax* and *P. ovale*. Chloroquine cannot prevent relapse because the dormant stage occurs in the hepaptocyte.[17]

For prophylaxis, chloroquine should be started one to two weeks before traveling to the malaria endemic area. While in the endemic country the drug should

be taken on the same day of the week once a week. After the traveler returns, he/she should continue taking chloroquine for four weeks. Chloroquine is given as a tablet of 500mg with 300 mg of the base. It may be used in pregnant women and children. For children the dose is determined by weight, the standard is 5 mg/kg with a maximum of 300 mg of base. Side effects of chloroquine include gastrointestinal disturbance, headache, dizziness, blurred vision, insomnia, and pruritus.[18]

Mechanism of Action

Chloroquine is classified as an aminoquinoline. The common mechanism of action of this class of pharmaceuticals is to inhibit the heme polymerase activity of the *Plasmodium*. The *Plasmodium* use heme polymerase to enzymatically convert heme, which is toxic to the parasite, to a hemazoin, which is nontoxic to the parasite. Therefore, the chloroquine activity of inhibiting the *Plasmodium*'s heme polymerase causes an increase of heme concentration in the red blood cell. It is expected that heme interrupts the biosynthesis of nucleic acids. Chloroquine binds to the heme that has accumulated to form the heme-chloroquine complex, which is highly toxic to *Plasmodium*. The heme-chloroquine complex interrupts the *Plasmodium* membrane. This causes lyses of the infected erythrocyte, which causes *Plasmodium* autodigestion.[19]

Chloroquine's highest concentration is found in the *Plasmodium*'s food vacuole. The medication is able to enter the *Plasmodium* by simple diffusion. The alkaline nature of chloroquine is important in the food vacuole, because it raises the pH of the vacuole. This rise in pH causes denaturing of the pigment protein, which

results in clumping. The rise in pH inhibits digestion of the amino acids the parasite acquires from degrading the hemoglobin of the host's red blood cells. Thus, chloroquine prevents *Plasmodium* from producing protein and creating energy that is needed for survival.[19]

Resistance

P. falciparum and *P. vivax* have chloroquine-resistant strains. Chloroquine was very successful when it was first approved in 1934 for treatment of malaria. Its early effectiveness resulted in heavy use of the medication at high doses. There were other antimalarial pharmaceuticals, but none had the low cost and high effectiveness of chloroquine. This ultimately led to resistant strains. *P. falciparum* and *P. vivax* are the most the common types of the parasite; therefore, they have the most exposure to chloroquine, which is why they have developed resistance.[11] *Figure 3* shows the areas of known chloroquine resistance in blue.

The first cases of resistance in *P. falciparum* were observed in Colombia and along the Thailand-Cambodia border in the late 1950s. In the1960s, resistance was noticed in parts of Southeast Asia, South America, and India. Cases of resistance in Africa were not identified until 1978, and those cases were initially noticed in Kenya and Tanzania, which are located on the eastern Africa sea border, near the equator. Within ten years of finding the resistant strains in Kenya and Tanzania, the *P. falciparum* chloroquine-resistant strain spread to the rest of Africa.[11]

The resistance found in *P. falciparum* has been found to correlate with a K76T (substitution for lysine with thyroxine at codon 76) point mutation of the gene

pfcrt (on chromosome 7). In fact, the K76T mutation is found in all resistant strains isolated and not in the sensitive strains isolated in the study performed by Djimde *et al.*[4, 20] PfCRT, the gene product, is a transporter that is found in the food vacuole membrane that regulates drug efflux and pH regulation. It is believed that the *P. falciparum* resistance to chloroquine is due to the increased efflux of chlororquine out of the food vacuole caused by the mutation. Seven other point mutations have been associated with chloroquine resistant strains of *Plasmodium*: M74I, N75E, A220S, Q271E, N326S, I356T, and R371I.[11] This mutation does not allow chloroquine to accumulate in the parasites food vacuole, thus, blocks the inhibition of the heme polymerase mechanism of the drug.[21] The rate of expulsion of chloroquine in resistant strains is 30 to 40 times greater than strains that are sensitive to chloroquine.[3]

Another mutation *pfmdr*-1 and -2 also causes resistance to chloroquine. *Pfmdr*-1 is located on chromosome 5 and codes for P-glycoprotein homologne-1 (*Pgh*-1). The mutation is a point mutation at codon 86 that switches aspartic acid for tyrosine. There are other mutations that have been shown to cause mutation, such as Phe 184, Cys 1034, Asp 1042, and Tyr 1246.[4]

Calcium channels blockers and other medications cause the efflux mechanism of the parasite can reverse the resistance of *P. falciparum* strains to chloroquine. The medications commonly used are verapamil and dilitazem.[4]

P. vivax resistance to chloroquine was not discovered until 1989 in Papua New Guinea. Resistance has spread to parts of South America and Southeast Asia.[11]

Figure 3

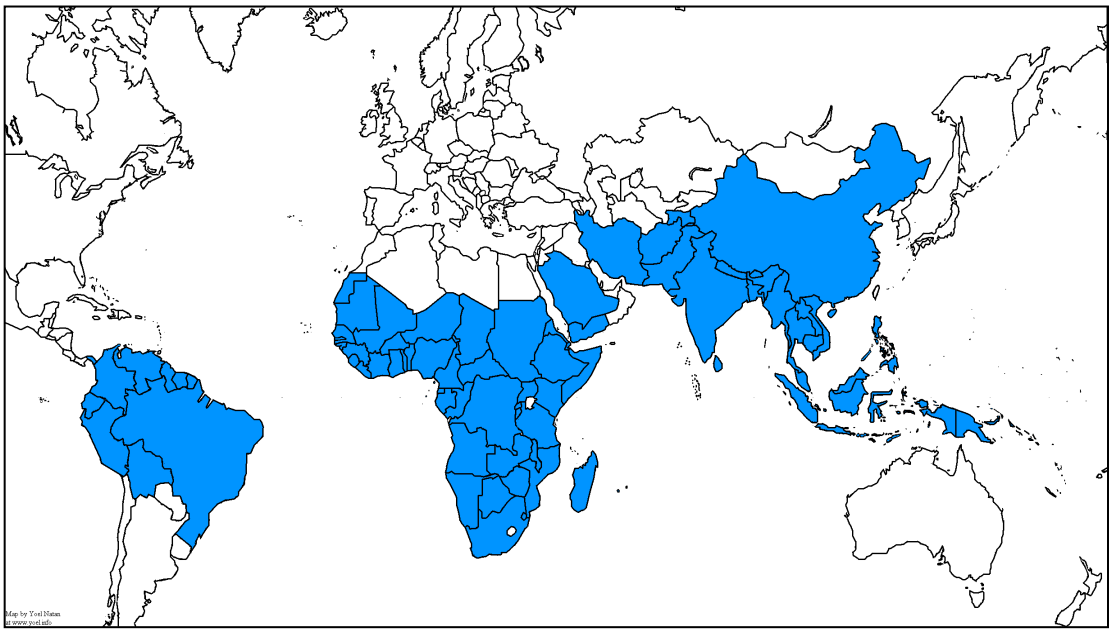

Figure 3. This is a map of documented *Plasmodium* resistance to chloroquine. Data was complied using Centers of Disease Control and *Guidelines for the Treatment of Malaria.*[22, 23]

Hydroxychloroquine

Hydroxychloroquine has the same structure as chloroquine with an additional hydroxyl group. The additional hydroxyl group does not cause any clinically significant pharmacokinetic differences between hydroxychloroquine and chloroquine according to a study by Tett, Cutler, Day and Brown.[24] Therfore, hydroxychloroquine cannot be used in areas with known chloroquine resistance.[25]

For prophylaxis, hydroxychloroquine uses the same schedule as chloroquine. The dose is 400 mg tablet with 310 mg of the base. The maximum dose is one tablet. Pediatric patients should be given 5 m/kg.[18]

Mefloquine

General Drug Information

Mefloquine, an analog of quinine, is used to treat and prevent malaria. Mefloquine's chemical structure is 2- piperidinyl-2,8- bis (trifuoromethyl)-4-quinolinemethanol hydrochloride. Mefloquine is used for the prevention and treatment of *P. falciparum* and *P. vivax*. The medication is also effective against cholroquine-resistant strains of *P. falciparum*.[26] It is also active against the gametocytes of *P. vivax*, *P. ovale*, and *P. malariae*. The destruction of gametocytes prevents further spread of the parasite to other mosquitoes and humans.[27]

For prophylaxis, a traveler should start taking mefloquine at least two weeks before travel. It is taken weekly on the same day of the week while in the malaria endemic country and four weeks after the traveler has returned home. For adults, mefloquine should be given as a 250 mg tablet with 228 mg of the base. It may be used for pediatric travelers. Children that weigh less than 9 kg should take 4.6 mg/kg. If they weigh between 9 and 19 kg they should take ¼ of the adult tablet. For patients weighing between 19 and 30 kg, they should be given ½ of the adult tablet. If they weigh between 30 and 35 kg, they should be given ¾ of the adult tablet. For children weighing more than 45 kg, they should be administered one full adult tablet. Common side effects include gastrointestinal disturbance, headache, insomnia, abnormal dreams, visual disturbances, depression anxiety, and dizziness.[18]

Mechanism of Action

Mefloquine acts on the erythrocyte stage *Plasmodium*; however, the medication is not effective against the exo-erythrocytes stages of *Plasmodium*. Thus, it is not effective against the dormant phase of *P. ovale* and *P. vivax*. The exact mechanism is unknown.[25] In *P. falciparum*, melfloquine may cause swelling of the food vacuole. Mefloquine is suspected to bind with heme to generate toxic complexes, which then damage parasitic membranes and disrupt other cellular components.[28]

Mefloquine has also shown cross-resistance with halofantrine, which is an antimalarial drug used against erythrocyte stages. In patients that are receiving halofantrine and were previously treated with mefloquine, the Q-T interval duration has been increased to the point of causing cardiotoxicity. [24]

Resistance

Resistance to mefloquine is present within strains of *P. falciparum*. Resistant strains are found in Southeast Asia and the Amazon region of South America.[22] Mefloquine resistance has been associated with an increase in the number of copies of the *pfmdr-1* gene.[4] *Figure 4* shows areas of known mefloquine resistance.

Figure 4

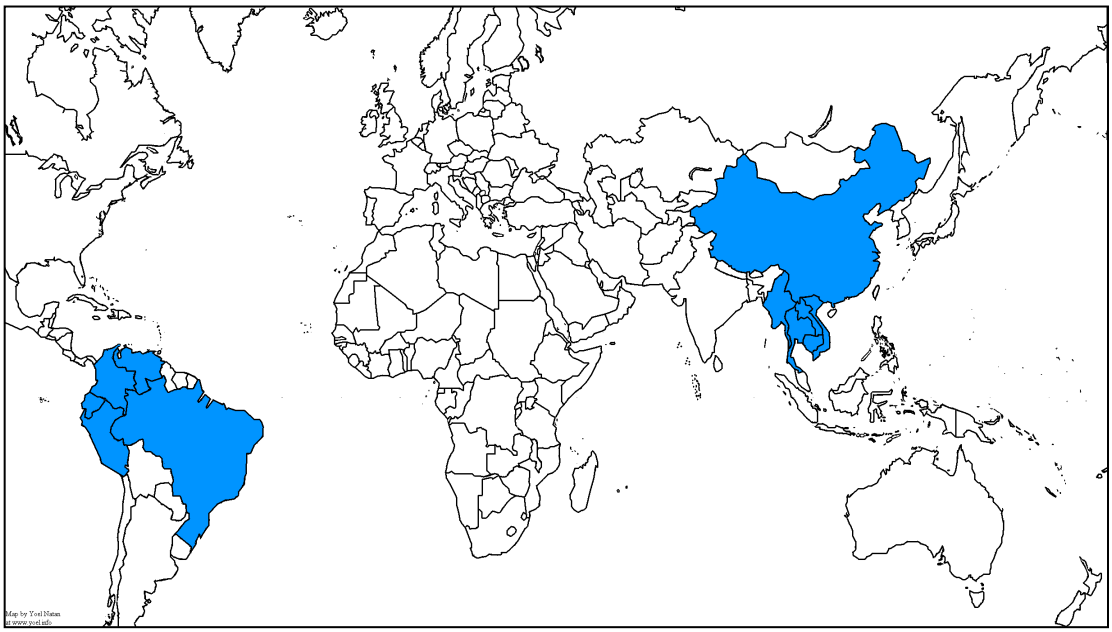

Figure 4. This is a map of documented *Plasmodium* resistance to mefloquine. Data was complied using Centers of Disease Control and *Guidelines for the Treatment of Malaria.*[22, 23]

Quinine sulfate

General drug information

Quinine sulfate is an antimalarial agent that acts on the erythrocyte stages of *Plasmodium*. The medication alkaloid derived from the bark of the cinchona tree. The use of quinine sulfate for the treatment of malaria has declined since the discovery of chloroquine, which is less toxic than quinine. Now, quinine is only used for malaria caused by multiple-drug resistant strains of *Plasmodium.*[25]

For treatment of malaria, quinine sulfate should be administered every eighth hour for seven days. The standard dose for pediatric and adult patients is 648 mg.

Most common side effects include diarrhea, nausea, gastrointestinal pain, and vomiting.[29]

Mechanism of action

Quinine sulfate is a blood schizontocidal agent meaning it kills the schizont phase of erythrocyte stage.[29] The precise mechanism of quinine sulfate is not known.[30] Quinine inhibits nucleic acids, protein synthesis, and glycolysis of *P. falciparum*. It also inhibits the parasite's mechanism of detoxifying heme by binding to the chemical used to convert hemozoin.[30]

Resistance

Plasmodium resistance to quinine has been reported in areas of South America, Southeast Asia, and Bangladesh.[29] The first report of the resistance was in Brazil about 100 years ago. The resistance developed in Southeast Asia in the 1980s because of the increased use of quinine.[23] Like chloroquine and mefloquine, *pfmdr-1* mutations are associated with quinine resistance.[4] The known pattern of quinine sulfate resistance is shown in *figure 5*.

Figure 5

Figure 5. This is a map of documented *Plasmodium* resistance to quinine. Data was complied using *Guidelines for the Treatment of Malaria.*[23]

Chapter V: Tissue Schizonticides

Pyrimethamine and sulfadoxine

General drug information

Pyrimethamine and sulfadoxine are used synergistically to inhibit dihydrofolate reductase and dihydropteroate synthase. It works in the erythrocyte stage of *P. falciparum* and *P. vivax*. The combination product may be used in areas where there is chloroquine resistance.[28]

Mechanism of action

The pharmaceutical drug class of antifolates work by blocking two of the key enzymes needed for the synthesis of folate sequentially and synergistically. Pyrimethamine inhibits dyhydrofolate reductase (DHFR). Sulfadoxine inhibits dyhydropteroate synthase (DHPS).[4]

Resistance

Resistance is caused by gene mutation to both DHFR and DHPS. The resistance occurs at varying degrees.[4] Resistance has been seen since the 1950s, about

ten years after the drug had been introduced as treatment for malaria. High-levels of resistance have been found in South-East Asia, southern China, and the Amazon region of South America. Lower levels of resistance are seen in Africa, Oceana, coastal areas of South America and southern Asia.[31]

Point mutations of *dhps* affect the efficacy of sulfadoxine of the combination product. The mutations are point mutations that occur on five codons: substitution of alanine or phenylalaine for serine at codon 436, glycine for alanine at codon 437, glutamic acid for lysine at codon 540, glycine for alanine at codon 581, and serine or threonine for alanine at codon 613. The mutation at 437 and 540 may occur together.[4]

Pyrimethamine resistance is associated with a point mutation on the *dhfr* gene. These mutations occur at codon 16 (substitution of valine for alanine), codon 51 (isoleucine for aspargine), codon 59 (arginine for cysteine), codon 108 (asparginine for serine), and codon 164 (leucine for isoleucine and threonine). The main mutation for pyrimethamine resistance is the substitution of asparginine for serine.[4] *Figure 6* shows the pattern of pyrimethamine and sulfadoxine resistance.

Figure 6

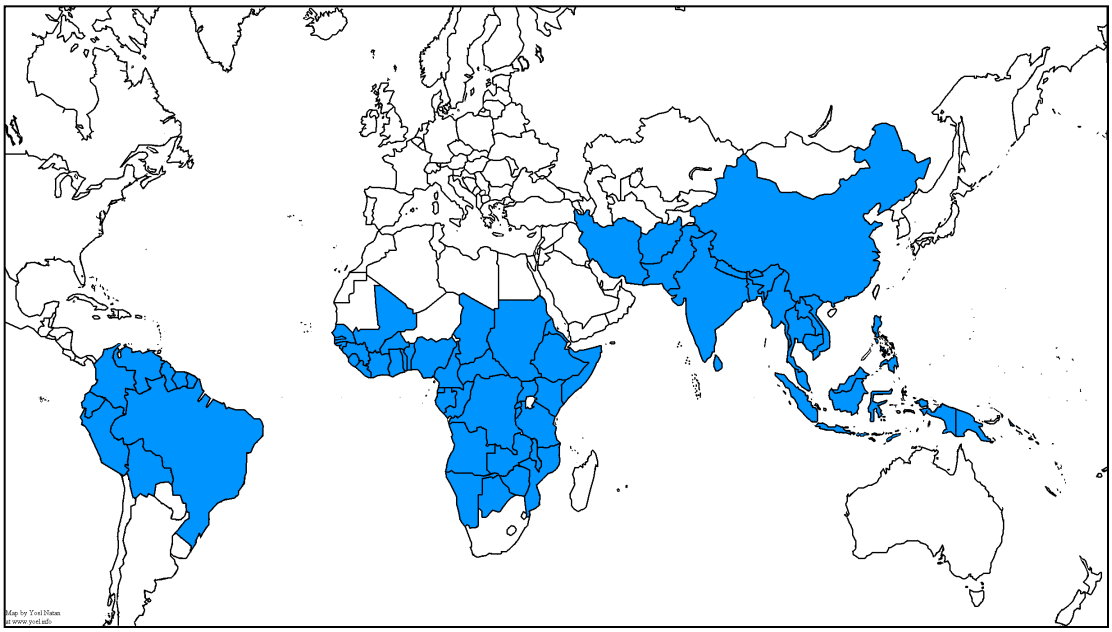

Figure 6.This is a map of documented *Plasmodium* resistance to Pyrimethamine and sulfadoxine . Data was complied using Centers of Disease Control and *Guidelines for the Treatment of Malaria.*[23]

Doxycycline

General drug information

Doxycycline is a synthetic derivative of oxytetracycline used as a broad-spectrum antibiotic. Doxycycline must be used in combination with other antimalarial pharmaceuticals to be effective.[32] The effectiveness of doxycycline as an antimalarial pharmaceutical depends on the patient's adherence to the medication.[32] Even though doxycycline is an antibiotic, it can be used for prophylaxis of malaria for travelers who are visiting areas with known chloroquine and/or pyrimethamine-sulfadoxine resistant strains of *P. falciparum*. For prophylaxis, it is recommended that a person traveling to countries that have high malaria endemic rates start administration at least a day before traveling, then one dose for every day that the traveler is in the country,

and 28 days after the traveler has returned home. The regular dosing is 100 mg per day for adults.[4]

Doxycycline can be used in combination with quinine or other rapidly acting schizonticide. Doxycycline with quinine improve the efficacy of treatment in areas known chloroquine resistance strains of *P. falciparum.*[32]

Mechanism of action

Doxycycline works in the erythrocyte stage. In studies, doxycycline destroys the schizont slowly. This medication shows no indication of sporocyte or gametocyte destruction. Doxycycline is metabolized in the liver.[32]

Resistance

There have been cases of resistance to doxycyline. The cases occurred because of inadequate serum concentration levels of doxycycline, which is most likely due to non-adherence.[32]

Atovaquone and proguanil (Malarone®)

General drug Information

Atovaquone and proguanil hydrochloride are manufactured in combination as Malarone® for the prophylaxis and treatment of acute, uncomplicated malaria caused by *P. falciparum*. Malarone® has been observed to be effective in areas of chloroquine- and mefloquine-resistant strains.[33]

For prophylaxis, atovaquone and proguanil hydrochloride therapy should be started one to two days before travel. It should be taken every day at the same time of day. The therapy should be taken while in the malaria endemic country and for seven days after leaving the endemic country. The adult tablets are 250 mg of atovaquone and 100 mg proguanil hydrochloride. It is not recommended for children less than 5 kg and pregnant women. The pediatric tablet is 62.5 mg of atovaquone and 25 mg of proguanil hydrochloride. For children that weigh between 5 to 8 kg, should take ½ pediatric tablet as standard dose. Children weighing between 8 to 10 kg, take ¾ tablet daily. For children weighing between 10 to 20 kg, they should take one pediatric tablet. Two pediatric tablets should be given to children weighing between 20 to 30 kg. For children weighing between 30 to 40 kg, they should be administered 3 pediatric tablets. One adult tablet should be given for those weigh more than 40 kg. Common side effects of atovaquone and proguanil hydrochloride are blurred vision, insomnia, dizziness, headache, gastrointestinal disturbance, and pruritus.[18]

Mechanism of action

Malarone® interferes with the pathways of *Plasmodia*'s synthesis of nucleic acids. Atovaquone selectively inhibits the mitochondrial electron transport at the cytochrome *bcl* complex of the parasite, which stops ATP synthesis.[4, 34] Proguanil hydrochloride disrupts the parasite's deoxythymidylate synthesis by inhibiting the dihydrofolate reductase inhibitor.[34]

Resistance

Resistance to atovaquone is caused by a single-point mutation in the cytochrome-b gene (cytB).[4] However, there is no known resistance to proguanil, which is thought to be the more important component of this combination.[23]

Chapter VI: Hypnozoitocides

Primaquine

General drug information

Primaquine phoshate, 8-[(4-amino-1-methylbutyl)amino]-6-methoxyquinoline phosphate, is an antimalarial pharmaceutical that inhibits the relapse of *P. vivax*. It was the first and only pharmaceutical discovered that would terminate the exo-erythrocyte stage of *P. vivax* and *P. ovale*. Primaquine is typically only used for treatment. This mediation is recommended as a radical cure and prevention of relapse of malaria caused by *P. vivax,* and for use in areas of endemic malaria caused by *P. vivax*. Primaquine is recommended after the termination of chloroquine administered as suppressive therapy.[35]

If a case of *P. vivax* malaria occurs, it is recommended to take primaquine along with chloroquine. Chloroquine eliminates the erythrocyte stage of the parasite and terminates the paroxysm. To destroy the parasite in the exo-erythrocyte stage, primaquine should be administered concurrently with chloroquine.[35]

For prophylaxis, primquine therapy should be started one or two days before traveling to malarious areas. The medication should be taken at the same time

everyday while in the endemic country and for seven days afterwards. The adult dose is 30 mg of base in 52.6 mg of salt. Pediatrics should take 0.5mg/kg of base with a maximum dose of 30 mg of base. The most common side effect is gastrointestinal upset.[18]

Mechanism of Action

The exact mechanism of action of primaquine is not known. It is suspected that primaquine may act by producing a reactive oxygen species or by disrupting the electron transport of *Plasmodium*. Primaquine may change the DNA of the parasite.[36]

However, the basic effects of primaquine towards *Plasmodium* are known. Primaquine inhibits the parasite from converting heme to a non-toxic agent by interfering with the mitochondria. Primaquine's interference with the parasite's mitochondrion inhibits the production of ATP, which the parasite needs to generate the chemicals to detoxify the heme. Thus, the parasite is eliminated.[36]

In the exo- erythrocyte stage, primaquine kills the intrahepatic form of *P. vivax* and *P. ovale*. This mechanism of primaquine prevents the relapse of malaria that is associated with *P. vivax* and *P. ovale.* Also, primaquine eliminates all forms of *Plasmodia* gametocytes preventing the spread of malaria by the *Anopheles* mosquito.[36]

Resistance

The main concern with resistance is that primaquine is the only pharmaceutical that terminates the hypnozoite form of *P. vivax* and *P. ovale* and

prevents relapse. The resistance to primaquine occurs in the elimination of the *P. vivax* in the exo-erythrocyte stage. The resistance is primarily due to lower dosing of primaquine, a shorter duration of treatment, and patient non-compliance.[37]

The resistance due to a lower dose than recommended primarily happens because of the adverse effects of primaquine at higher doses and/or the miscalculation of dosage for an individual patient.[37] The normal dose given is 15 mg of primaquine for 14 days to fully eradicate the exo-erythrocyte stage of *P. vivax*.[35] Primaquine is more effective at higher doses than recommended 15 mg. However, the adverse effects of primaquine are dose related. The dosing of primaquine is based on the patient's weight. If too low of a dosage is calculated, it will be ineffective. If the duration of treatment with primaquine is shorter than recommended, primaquine also becomes ineffective and allows the parasite to develop resistant strategies to the drug.[37]

Non-compliance by the patient is another issue that causes primaquine resistance. With the recommended 14-day treatment of primaquine, the patient may stop taking the medication after the illness symptoms resolve, especially if the patient suffers from any adverse effects from the medication. This would, in turn, allow *P. vivax* the opportunity to develop a resistance mechanism against the drug.[37]

Chapter VII: Artemisinin

Arthemether and lumenfantrine

General Information

The United States Food and Drug Administration approved the first artemisinin-based combination treatment (ACT), Coartem® (arthemether and lumenfantrine).[38] Coartem® is indicated for the treatment of acute, uncomplicated malaria infection due to *Plasmodium falciparum* in patients weighing more than 5 kg. It is not indicated for complicated malaria or the prophylaxis.[39] It has shown high effectiveness in areas of known multi-antimalarial resistance to treat malaria.[38] Each Coartem® tablet consists of 20 mg of arthemether and 120 mg of lumenfantrine. It is a 3-day regimen with doses 2 times a day. One dose consists of 4 tablets if the patient weighs more than 35 kg. For patients that weigh between 25 kg and 35 kg the dose should be 3 tablets. For patients that weigh between 15 kg and 25 kg the dose should be 2 tablets. For patients that weigh between 5 kg and 15 kg, the dose should be 1 tablet.[39]

Mechanism of Action

Together, arthemether and lumenfantrine inhibit nucleic acid and protein synthesis. Arthemether is metabolized predominantly by CYP450 3A4 and CYP450 3A5 into dihydroartemisinin, which is an active metabolite. Lumefantrine inhibits the formation of Beta-hematin by forming a complex with hemin.[40]

Resistance

The Center of Disease Control did not adopt arthremether and lumenfantrine as the first-line drug for treatment of uncomplicated malaria until 2004. Because this drug is relatively new, there are no confirmed regions of *Plasmodium* resistance.[41]

Chapter VIII: Future resistance

Malaria will continue to be a problem worldwide. *Plasmodium* will continue to develop resistance against antimalarial pharmaceuticals. The parasite develops resistance much faster than the rate of new pharmaceuticals is being developed. It is thought that the only way to prevent malaria and cases of resistance is find another way to treat the disease besides medications. The industry is working on developing vaccines, but the *Plasmodium* genus has so many different sequences it has proven very difficult to do.[4]

Most pharmaceuticals that are being developed are artemesinin-combined treatment, which are pharmaceuticals that have a form of artemesinin and another drug. It is hoped that these combination drugs will slow the rapid rate of *Plasmodium* resistance.[42] The World Health Organization (WHO) believes ACTs are the key, because the artemisinin component is highly active with only a short regimen. They rapidly clear the erythrocyte stage, which leads to the reduction of gametocytes that cause transmission. The second component has a longer acting effect. The WHO recommends five ACTs as first-line treatment against acute uncomplicated malaria. However, Coartem® is the only ACT available in the United States.[43]

Resistance has also been associated with low drug concentrations. The low drug concentrations occur because of poor adherence taking the medication, sub-standard drugs, or unusual pharamcokinetics. The patient may be non-adherent to the medication because of the common side effects associated with the antimalarial pharmaceuticals. One of the common adverse effects of the medication and symptom of malaria is vomiting, which can also lower the drug concentration in the patient. The pharmacokinetics of the pharmaceuticals can be altered by the function of enzymes that metabolize the drug. For example, artemether, which is a component of Coartem®, is metabolized into an active metabolite by CYP450 3A4 and 3A5. If the patient is a poor metabolizer of one of these enzymes, the concentration of the active metabolite will be lowered. This lower concentration allows the parasite to develop resistance.[44]

The CDC defines sub-standard antimalarial pharmaceuticals as "having no active ingredients, they may have less than the required amount of active ingredient, or they may contain ingredients which are not what is described on the package label." In poor countries, counterfeit drugs are often found with lower concentration of the active ingredients. The lower concentration of active ingredients allows the parasite to develop resistance. These countries tend to have very little or no pharmaceutical regulation or enforcement.[45]

Conclusion

Malaria has been around almost 4,000 years. The *Plasmodium* genus is responsible for the disease. *P. falciparum* and *P. vivax* are the most common species that cause the disease. The parasite needs two hosts to complete the life cycle, the *Anopheles* mosquito and humans.

Symptoms can occur in a tertian or quartan cycle. Symptoms occur with the erythrocyte stage of the plasmodium. The most severe complication is severe cerebral malaria caused by *P. falciparum.*

The sickle cell trait enhances a person's protection against malaria. The trait occurs at a higher frequency in areas that are known as malaria endemic.

Plasmodium have developed resistance to chloroquine because the over-use at high doses of chloroquine. The *Plasmodia* develop resistance through single amino acids mutations. Quinine sulfate and mefloquine have also caused *pmfdr* mutations in the *Plasmodium* genome that have resulted in the resistance. Malarone® resistance is due to cytochrome-b mutation that effects the atovaquone component's action. There are mutations that cause resistance to both components of pyrimehtamine and sulfadoxine. Primaquine resistance has been attributed to mutations caused by poor

adherence by patients to the medication or to a miscalculation of dosage that should be given to patient.

Today, *Plasmodium* develops resistance to the antimalarial medications faster than the pharmaceutical industry can develop antimalarial medication. The main cause of resistance is lower concentrations of the active ingredient of the antimalarial pharmaceuticals caused by lack of compliance, vomiting, sub-standard drugs, and/or pharmacokinetics. Resistance will continue to be a problem associated with antimalarial medications. The pharmaceutical industry must develop another way besides medications to prevent malaria, otherwise resistance will continue.

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