Clinical Microbiology and Infectious Diseases

Review Article

ISSN: 2398-8096

Emergence of drug resistance in *Plasmodiun falciparum*: Reasons of its dispersal and transmission in different climatic regions of the world: a review

Ravi K. Upadhyay*

Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur-273009, U.P, India

Abstract

In the present time emergence, dispersal and transmission of drug resistant malaria parasite *P. falciparum* has become a serious health problem in human being throughout the globe. From various surveys it has been proved that intensity of drug resistance and pathogenesis of dreadful parasite is increasing day by day due to arousal of point mutations in dhfr and dhps genes mainly drug binding regions of *P. falciparum* genome. However, single or their multiple point mutations have altered the allele frequencies in *P. falciparum* clinical isolates collected from various regions of the world that resulted in emergence of drug resistance and lead to complete failures of anti-malarial drugs. Further it has increased dispersal and transmission of drug resistant *P. falciparum* throughout Africa and Asia. Genetic reasons of drug failures the intensity of parasite survival and its resistance to various drugs seems to be widely influenced due to climatic and demographic reasons mainly rapid and active breeding of disease transmission vectors, poor health hygienic conditions, use of substandard diagnostic facilities and low grade treatments provided to the patients. In addition, human migration and poor rehabilitation have enhanced the severity and complications of malaria and its seasonal outbreaks. Therefore, for fast control of malaria, high quality diagnostic and treatment facilities are required for better therapeutic results to fight against deadly *P. falciparum* outbreaks.

Introduction

Malaria is a dreadful infectious disease and has become a major impediment to socio-economic development in Africa, Asia and other poor nations of the world. Today it has become a global burden as an estimated 359 million cases are reported every year and 1.5-2.0 million deaths annually globally. Most of these deaths are largely concerned to the African countries [1]. Recent emergence of resistance to both old and new anti-malarial and its subsequent spread to non-infecting areas undoubtedly make the situation more terrible. Intervention by WHO and other malaria controlling agencies/institutions, it still exists as endemic diseases in densely populated South-East Asian and Sub Saharan African countries. In both the regions malaria became highly problematic due to eruption of multi-drug resistant *P. falciparum* mutants. Few countries, like Bangladesh, Myanmar, Philippines, Thailand, Cambodia, Eastern India, Indo-Nepal border, and Myanmar-China border become the breeding ground of multi-drug resistant *Plasmodium falciparum*. Recent detection of ACT resistance in *P. falciparum* has made the situation more alarming. However, due to long term over and repetitive use of antibiotics, malaria parasites have become resistant to most of them. It has further reduced the drug efficacy and increased the drug dose/level mainly IC_{50} values manifold. Subsequently, it has resulted in an increased rapid dispersal and transmission of drug resistant falciparum malaria [2]. Their drug-resistant pfdhps haplotypes are circulating in West Africa and many Asian countries. Therefore, after seeing the rapid spread of multi-drug resistant *P. falciparum* mostly in poor countries of the world, it become mandatory to design new anti-malarial drugs with new viable strategies to check the emergence and spread of future drug resistance. All new alternative drugs need to be tested for their efficacy [3] to control high infection rate acquired by malaria parasite [4]. Besides this, there is a vast difference in drug sensitivity of parasites in many regions and it varies from region to region [5]. In some of the pockets same drug is thought to be effective but again it has no effect in other neighboring country. All it is due to increased drug pressure that has induced genomic changes mainly in dhfr and dhps genes at regional level. Therefore, there is an urgent need to collect molecular epidemiological information from different countries for quick analysis of data to know all possible reasons of origin and spread of drug resistant malaria [6].

Spread of multidrug resistant malaria in Asia and Africa

Due to demographic, eco-climatic and genetic reasons multidrug resistant malaria is widely spreading in Asia and Africa and rest of the world [7]. Mainly mutations occurred in dhfr and dhps genes conferred high levels of resistance in malaria parasite. It has increased density of malaria parasite in patients; hence, malaria treatment has become very difficult [8]. These genetically resistant infectious strains of *P. falciparum* malaria are reported from many countries of the world such as Mali [9], Sub-Saharan Africa [10], Somalia [11], Thailand [12], Mozambique [13], Rwanda [14], Swaziland [15], Soloman islands [16], Iran [17], Nigeria [18] and Kenya [19]. Besides this, Trimethoporin

Correspondence to: Ravi K. Upadhyay, Department of Zoology, D.D.U. Gorakhpur University, Gorakhpur-273009, U.P, India, **E-mail:** rkupadhya@yahoo.com

Key words: *malaria, P. falciparum, drug resistance, pyrimethamine-sulfadoxine, chloroquine*

Received: July 06, 2016; **Accepted:** July 31, 2016; **Published:** August 04, 2016

sulfamethoxole resistance mediating dhfr 16LL mutations have been detected in Ugandan population [20]. CQ mefloquine, quinine and SP/pyrimehtamine susceptibility in Somalia [21], Sierra Leone [22], Venezuala [23], Nigeria [24], Zambia [25], Phillipines [26], Zambia [27] and Thailand [28], while mefloquine [29], Proguanil/sulfamalaria CQ and pyrimethamine-sulfadoxine resistance was detected in Nigeria [30], CQ resistance in Kenya [31] and Diaoluo area in Hainan province of China [32]. Chloroquine resistant *P. falciparum* was identified in indigenous residents of Cameroon [33], Kenya [34] and Nigeria [35]. Similarly, few old prescriptions like fanisder-sulphate and quinine and fanisder-HCl tetracycline [36], proguanil/sulfamethoxazole and sulfalene+pyrimethamine are also used to cure *P. falciparum* malaria in many African and Asian countries but these have totally failed to fight against drug resistant malaria parasite [37]. Non-artimisinin and artimisinin based combination therapies are used to cure uncomplicated falciparum malaria patients [38]. But, again combination therapies fail to provide good results due to emergence of resistance in *P. falciparum* [39]; hence, malaria control becomes very difficult and seems to be impossible [40] because of genetic and statistical complexity of the parasite mutation (Table 1) [41].

Molecular basis of resistance to a number of common anti-malarial drugs is well known, but epidemiological reasons of emergence and dispersal of drug resistant mutations in *P. falciparum* in many Asian and African countries are not fully known. Few strong reasons which have been identified for transmission of multi drug resistant malaria are human trafficking/traveling to malaria endemic to epidemic regions. Problem of human migration and instability created due to cross border tensions, paved way for the establishment of large refugee camps devoid of sanitation, diagnostic and treatment facilities. These refugee camps become the epicenter of drug resistant *P. falciparum* strains and work as reservoir of parasites. Such regions are mainly present in eastern Afghanistan where refugees crossed into the federally administered tribal areas of northwestern Pakistan. When these camps were monitored they have shown very high malaria incidence as 100.4 cases/1,000 person-years (Table 1). Hence, proper diagnosis and better treatment is required for fast control of malaria in such regions [42]. Similar cases of malaria were detected in asymptomatic children in malaria endemic sites in Western Kenya [43]. It happens due to human travel that enhances prevalence, genetic variability and rate of gene mutations in *Plasmodium falciparum*. Blood bites made by local female mosquito vectors on non-resident mainly infected travelers and tourists further enhance the transmission rate of drug resistant malaria in non-drug resistant population. It is also responsible for shaping current parasite population structure having multiple mutations [44].

Reasons of spread of drug resistant malaria in India

In India after launch of the National Malaria Control Programme in 1953, the number of malaria cases reported in India has sharply declined. After 1965 number has been enormously increased due to spread of drug resistant malaria in different parts of the country. In spite of sound efforts made by ICMR and malaria control board, no initial success was achieved against resurgence of malaria since 1960 and every year thousands of people died due to malaria mainly infants below age of 5 years. In India first time chloroquine resistance in *Plasmodium falciparum* was reported in 1973. Since then, infectivity has increased manifold with time due to rapid urbanization, human migration and poor housing establishments in the vicinity of water reservoirs having active breeding of malaria vector. Low control of vector, poor diagnosis and faulty medication lead to increase in transmission rate of resistant *P. falciparum* and almost every year outbreak of malaria epidemics occurred in many parts of India [45] like Orissa, Assam, West Bengal and other north eastern states of the country [46]. Similar resurgence of malaria and chloroquine resistance in *P. falciparum* and *P. vivax* was reported from Bombay, India [47], all along Indo-Nepal border [48] and western Myanmar [49]. Hence, for good therapeutic outcomes [50] regular consultation, proper diagnosis and appropriates prescription [51] of the anti-malarial drugs are essentially required [52] to cure regional and imported malaria cases [53]. Besides this, spectrum of new anti-malarial drugs must be evaluated from time to time.

Genetic analysis of *P. falciparum* parasite

For determining the level of resistance and transmission, genetic and molecular analysis of malaria parasite is important. Few important tools like micro-satellite methods are used to show the presence of multiple lineages for the mutant dhfr genotype (Table 1) [54]. However, on the basis of number of mutations occurred in parasite, the level of drug resistance can be predicted in clinical isolates. Further, drug use

Table 1. Showing drug combination used and country wise per cent allele frequencies obtained in *P. Falciparum* malaria parasite.

Drug/Drug combination	Country	Allele	Frequency (%)
Chloroquinine-Sulfadoxine/ Pyrimethamine	Malo Island	pfcrt	95.4
Sulfadoxine	Cambodia	pfcrt	17.9
Sulfadoxine/Pyrimethamine	Costal Kenya	dhfr	41
Chloroquinine-Sulfadoxine/ Pyrimethamine	Bangladesh	pfcrt	2.39
		pfmdr1	43.51
		dhfr	63.59
		dhps	98.8
Sulfadoxine/Pyrimethamine	Myanmar	dhfr	49.25
		dhps	92.7
Chloroquinine	Bangui (Central Africa)	pfcrt	0.6
		pfmdr1	78.1
		dhfr	21.6
		dhps	18.9
sulfadoxine-pyrimethamine	Peruvian Amazon	dhfr	0.2
		dhps	1.7
sulfadoxine-pyrimethamine	Tanzania	dhps	89.5
sulfadoxine-pyrimethamine	Africa	pfdhfr	75
sulfadoxine-pyrimethamine	India	dhfr	17.52
		pfdhf	2.89
		dhps	23.69
sulfadoxine-pyrimethamine	West Africa	pfdhps	11
sulfadoxine-pyrimethamine	Sudan	dhfr	45
		dhps	12.6
Sulfonamide/Pyrimethamine	Thailand	pfdhfr	3.89
	Pakistan	dhfr	28.5
		dhps	23.61
Tetracycline	Southern Mozanbique	dhfr	37.8
		dhps	72.1
Clotrimoxazole	Uganda	dhfr	21
Amodiaquine	Uganda	pfmdr1	72
Sulfonamide/Pyrimethamine	Rwanda	pfdhfr	61.4
		pfdhps	28.8
Chloroquine	Zambia	pfcrt	58.3
Sulfadoxine /Pyrimethamine	Zambia	dhfr	26.4
Chloroquine	Senegal	dhps	89.7
Mefloquine-Sulfadoxine-Pyrimethamine	Nigeria	pfdhfr	29.1
Chloroquinine-Sulfadoxine/ Pyrimethamine	Philippines	pfcrt	87.5

and its efficacy (ID50 value) are directly connected with mutations. If there is a single mutation then drug may be active or not, it cannot be decided by considering single factor in mind, but if any clinical isolate shows two or more mutations, it means there may be higher resistance is present in *P. falciparum* against multiple anti-malarial drug. There is a possibility that moderately mutated parasite with one mutation in DHPS genes may provide moderate asymptomatic parasitological failures of drugs in malaria patients [55]. Active immunity in malaria patients also work against *P. falciparum* infection. There are few malaria hot spots where mutant allele frequencies in pfdhfr are very high and malaria is out of control because patients have used multiple drugs against which parasite has already acquired resistance. Therefore, it is very hard to establish relationship between parasite genetics and *in vivo* treatment failure rates. Parasite floating in community has succeeded to establish genetic changes at regional level according to environmental conditions and both factors do influence genetic changes that is the reason why clinical and community samples collected from above sites have shown nearly similar allele and haplotype frequencies. Hence, predictions about success rates of anti-malarial drugs and clinical outcomes cannot be easily done. In order to determine drug efficacy and monitoring of drug resistance, high quality molecular markers must be required to make more appropriate decision about potential alternative of present anti-malarials [56]. All indicators based on molecular data need to be considered with caution and interpreted in the local context rather than as a large area. In addition to it, community data may also be affected by prior drug usage and level of pre-existing immunity in patients. It is achieved by a time different recombination rates among parasites which contribute drug selection used by various population groups that influence gene frequencies and drug resistance in malaria parasite [57]. However, low level of parasitimia is an indication of drug resistance and presence of mixed infection [58]. It is also concerned to *P. falciparum* chromosomal mutations [59] and polymorphism occurred in pfcrt, dhfr and dhps genes [61]. Ineffective low dose treatment combination resulted in origin of mixed parasite with sensitive DHFR genotype sensitive isolates [62] while longer use of high drug dose level establishes more resistance and non-sensitive DHFR genotype (Table 1) [62].

Origin of point mutations in drug resistant genes

Point mutations in dhps and dhfr genes are responsible for formation of various drug resistant mutant alleles of *P. falciparum* [63]. Allelic exchanges occurred at the endogenous genomic locus in *P. falciparum* caused genetic variabilities [64] that determine the drug resistance in a particular area [65]. To find every minute difference among drug resistant *P. falciparum* parasite MSP, MSP_2 and glutamine-rich protein typing is also used [66]. Besides this, polymorphism in Merozoite surface protien1 and 2 and the glutamate rich protein (GLURP) genes are used as genetic markers for the genotyping of field population of *P. falciparum* [67] mainly resistant SMPs haplotypes of dhfra and dhps genes [68]. Similarly, quartet mutations in dhfr/dhps genes were identified in clinical isolates isolated from New Papua New Guinea [69] and different regions of India (Uttar Pradesh, Madhya Pradesh, Assam, Orissa, and Andaman and Nicobar Islands) where malarial transmission rates and levels of drug resistance vary across the region. Among the isolates, a significant reduction in genetic variation in the +/-20-kb vicinity of the mutant pfdhfr alleles due to hitchhiking was observed. This reduction in genetic diversity was more prominent around quadruple pfdhfr alleles than around double and single mutant alleles [71]. Similar pfdhfr triple mutants were also identified in Thailand and other Southeast Asian countries [71]. Hence, control of multidrug resistant *P. falciparum* malaria [72] become very difficult because abundance of symptomatic carriers, reduced effectiveness of the available anti-malarial drugs and transmission of infection by highly adapted and pesticide resistant local mosquito strains in endemic regions (Table 1) [73].

Tetracycline resistance

Tetracyclines are used as first line treatment to cure malaria patient's worldwide. But it has been discontinued because of high prevalence of resistance acquired by malaria parasite. Therefore, for regaining therapeutic status, new and more active antibiotics are to be developed to strike upon malaria cases. In this category Tigacycline, a third generation tetracycline possesses broader spectrum activity was found to be good alternative for the treatment of complicated infections. But, due to very high toxicity and rate of resistance shown by malaria parasite [74] this drug is also banned and its use is being made very limited [75] that resulted in low infertility of *P. falciparum* gametocytes to *Anopheles gambiae* [76] and enhances the rate of gametocyte carriage [77]. Due to accumulation and high prevalence of mutations most of drug treatments become totally failed and proved useless [78]. Chloroquine resistance in *P. falciparum* is reported from India [79] while Clotrimoxazole (anti-foliate) resistance among persons infected with human immunodeficiency virus was reported in Eastern Uganda [80]. Similarly, 1246Y allele was found common in all field isolates collected from Bangui, Central Africa Republic (Table 1) [81].

Resistance to amodiaquine/sulphadoxine-pyrimethamine (AQ/SP)

Sulfadoxine-pyrimethamine or amodiaquine are commonly used in first line drug therapy to treat uncomplicated falciparum malaria cases [82]. But increasing therapeutic failures associated with the development of significant levels of resistance worldwide has forced to use alternative treatment regimens against malaria. But unfortunately malarial parasite has shown wider resistance to both Sulfadoxine-pyrimethrine (SP) and chloroquine (CQ) drugs. It has been spread rapidly within Africa mainly in Kenya where large portion of population is infected with Sulfadoxine- pyrimethrine resistant *P. falciparum* malaria due to rapid emergence of in pfdhps gene mutations [83]. Hence, there is a need to determine the factors related to adherence of amodiaquine/sulphadoxine-pyrimethamine (AQ/SP) and resistance grown in *Plasmodium falciparum*. It is spread across the continents due to high transmission mainly in community people [84]. Pyrimethamine shows high mutation rate in comparison to cycloguanil [85]. It enhances the degree of genomic polymorphism leading to diversity of natural parasite population [86]. SP. resistance in *P. falciparum* also shows deleterious effects in vitro on gametocyte infertility prevalence [87] and drug resistant highly infectious parasites [88].

The SDX- pyrimethamine resistance is caused after single point mutation occurred within the enzyme active sites [89] mainly dihydropteroate synthase (*dhps*) locus. It has shown independent origin of drug resistant alleles flanking the dhps locus [90] that has generated resistance to SDX, in *P. falciparum* [91]. It is an extremely rare mutation that has spread over large geographical areas of the world. Further, its subsequent spread has affected epidemiology at regional level that is an alarm for future [92]. Such isolates with point mutations in the dhfr and dhps genes of *P. falciparum* associated with pyremethamine and sulfadoxine resistance were also identified in India from Bikaner [93]. Majority of these isolates showed double mutant alleles for dhfr only in few cases. Recent surveys have revealed wide spread of a high-level pyrimethemine resistant lineage of *Plasmodium falciparum*, of Asian origin, across Africa from where it has shown some distinct genetic characteristics [94]. Undoubtedly, this lineage plays an important role in clinical failure to SP in Africa [95]. Similarly, non-responses gradient to SP and CQ were also found along Myanmar and India international border [96], which is probably indicative of the direction of the movement of the drug-resistant *P. falciparum* parasite (Table 1) [97]. Similar, cases of SP related uncomplicated *P. falciparum* malaria were found in Columbia [98] with an observed diversity of double and triple mutant alleles of dhps of a single origin. However, it can assumed that these multilocus genotypes including unlinked microsatellites loci were originated due to genetic exchanges taken place between low density parasite population and new migrants having malaria infected people [99]. Malaria patients from Rwanda have shown the highest levels of antimalarial drug resistance due to multiple resistances in pfdhfr genes and pfdhps mutations occur 1164L regions [100]. Similarly, increased prevalence of pfdhfr/phdhps mutants and drug resistance of *P. falciparum* is also reported in Kenya [101]. However, in the beginning of treatment parasite shows very low frequency but later on it enhanced enormously due to mutations occurred in the *P. falciparum* gene (dfhr) encoding dihydrofolate reductase aroused due to selection pressure [102]. Both activity and effectiveness of drug can be assumed by seeing the number of fever episodes and deaths prevented in children (Table 1) [103].

Genetic changes occurred in *P. falciparum* were also detected by using polymorphic microsatellite markers and its analysis [104]. With the help of this technology can explore origin and pattern of spread of drug resistant *P. falciparum* throughout world and can explore new independent lineages and routes of geographical spread of resistance. Further, comparison of molecular evolutionary analyses of samples collected from various endemic regions can identify existence of multi-lineage SP resistance in many endemic regions [105]. Therefore there is a need to collect molecular epidemiological information regarding dhfr and dhps genes for avoiding the widespread distribution of high levels of resistant parasite in non-infected human population [106]. Hence, an appropriate drug formula should be chosen to reduce the emergence and spread of future drug resistance [107]. Further, for studying the origin and evolution of drug resistance, microsatellite markers flanking the pfdhfr gene are to be mapped. Besides this, lactic dehydrogenase monitoring can be done in *P. falciparum* for screening therapeutic responses to standard malarial drugs (Table 1) [108].

Chloroquinine resistance

For eradication of *P. falciparum* malaria infection [109] both CQ and SP are predominant anti-malarial drugs of choice [110]. Both drugs showed high efficacy in patients and parasite clearance rate [111] that is why despite diminishing efficacy, chloroquine remains the primary anti-malarial agent in many endemic areas [112]. Emergence of CQ-resistance in *P. falciparum* is associated with a significantly higher prevalence of post-treatment gametocytaemia [113] and enhanced the lethality in malaria patients [114]. It is also widely concerned with accumulation of choloroquinine versus pyrimethamine/sulfadoxine resistant mutants in uncomplicated *P. falciparum* malaria cases [115]. In India chloroquine resistance in *Plasmodium falciparum* was first reported in 1973. It is caused due to rapid urbanization and civilian migration from infected to uninfected areas. In addition, intermixing of malaria infected patients with normal population and their migration to large geographical area caused very high transmission of malaria across the country [116]. A high degree chloroquine resistant P. falciparum was detected in Mandla districh (M.P.) India [117] and east Africa [118]. Similar, CQ-SP resistant Pfcrt alleles were detected in P. falciparum isolates that are responsible for seasonal out breaks of malaria on Malo island of Republic of Vanuatu [119]. Due to very high rate of transmission of chloroquine resistant malaria (CQ) led to its withdrawal from use in most countries like Malawi. But after a long gap of its withdrawal there was observed a rapid reduction in the frequency of resistance to the point mutations and the same drug is now considered to be effective once again. Such isolates need to be carefully examined by genetic markers to investigate the CQ-resistance against *Plasmodium falciparum* prior to the withdrawal of CQ. Hence, prior to an official ban poor molecular investigation of clinical isolates should be done properly and very carefully, because it may be a clinical fault [120] or may be due to immunity developed by patients in absence of drug [121]. To cure acute uncomplicated falciparum malaria extra care should be given [122]. Sometimes drug dose level [123] shows lesser efficacy due to irrelevant drug combination used against *P. falciparum* [124]. Hence clinical effectiveness [125] or therapeutic efficacy of both first line (chloroquine and amodiaquine) and second line drugs (sulfadoxime and pyrimethamine) must be prescribed very carefully [126] for successful management of uncomplicated *Plasmodium falciparum* infection occurred mainly in children [127] because multi-drug-resistant *P. falciparum* causes hematological malignancies in children [128]. Similar cases of sulfadoxine resistant falciparum malaria were detected along Thailand-Combodia border. It is real place for origin of CQ-PM resistance from where resistant strains were spread to Asian and African countries [129]. Similar cases of Q and SP resistant *P. falciparum* infection are reported in Soloman island (Table 1) [130].

Antifolate drug resistance

Due to increasing trends in chloroquine resistance the antifolate (Sulpfadoxine+pyrimethamine combination) drugs are used to treat of falciparum malaria [131]. Antifolate drugs primarily act as DHFR inhibitors and target folate biosynthesis in malaria parasite *P. falciparum* [132]. These drugs, (SP+PQ) in combination were found to be highly effective, safe and better tolerated to children and patients infected with drug resistant malaria [133] and showed superior efficacy than mono-therapies [134]. But due to long term anti-malarial monotherapy (MT) independent point mutations occurred in *P. falciparum* and *P. vivax* [135] both have attained antifolate resistance [136] and these novel pfdhps haplotypes are circulating in West Africa [137] and a mixture of wild-type and resistant pfdhfr and pfdhps alleles are also detected in tourist from this South-East Asian region [138]. Hence, there is an urgent need for the evaluation of alternative and affordable combination treatments (CT) for malaria patients [139]. In such cases both mefloquine [140] primaquine were found effective against *P. falciparum* up to some extent at early infection stage [141]. Besides this, to overcome drug resistance falciparum malaria fixed oral dose of artemisinin-naphthoquine combinations (ANQ, ARCO) can be used. These combination therapies provide safety, efficacy and tolerability to the patients. However, a single dose regimen of combination drug may be an effective treatment of uncomplicated *P. falciparum* malaria if regularly prescribed for three days (10 mg/kg/day) to adults [142]. Similarly PG-Ds could provide an effective affordable therapeutic alternative in East Asia [143]. But again such anti-malarial treatments provided in combinations are no longer found effective against *P. falciparum* [144].

Use of Artimisin-based combination therapies and resistance

Artimisin-derivative combination therapies (ACT) were found highly effective against multidrug resistant *P. falciparum* malaria than any other therapy used [145]. ACT is considered as a highly successful anti-malarial therapy that rapidly reduce both asexual and gametocyte stages of the *P. falciparum* life cycle [146]. It also reduces gametocyte carriage and infection rates in patients [147] and is potentially used for treatment of multidrug-resistant malaria in Africa [148] and Cameroon [149]. Similarly, in Vietnam, use of artemisinin derivatives provided initial high success in malaria control [150] but later on malaria parasite become highly resistant to them [151]. Similar cases of artimisin resistance concerned to sulfadoxine/pyrimethamine usage resulted in dhfr quadruple mutants i.e. pfcrt, pfmdr1, dhfr, and dhps in *Plasmodium falciparum* which were identified in clinical isolates collected from Myanmar and Bangladesh border areas (Table 1) [152] and also from Cambodia [153], where it is used to treat uncomplicated malaria [154]. Similarly, Artemether-Lumefanthrin (Coartem) and artesunate with sulfoxine-pyrimethamine therapy is also provided to uncomplicated malaria in Ethiopia that has also failed [155]. Hence, monotherapy for self-treatment should be avoided because inadequate treatment regimens favor emergence of drug resistance in malaria parasite [156].

Intermittent preventive treatment of malaria in infants

There is a serious problem to intermittent preventive treatment (IPTo) where drug combinations are provided to mother during pregnancy [157]. It is a promising malaria control strategy which is routinely used to cure the mother and her infant [158]. Use of (AQ/SP) combination raised many questions regarding high level of toxicity observed in clinical trials in context of family use. It shows a parasitological rebound effect due to an appropriate selection of drug and its clearance. But, recently, malaria parasite becomes resistant to IPTp and infections with mixed resistant and susceptible parasites get exacerbated [159]. During pregnancy severe malaria infection resulted in a low birth weight of infants [160]. If such mothers are not treated well, they usually pose high risk of miscarriages and also show weak prospective delivery [161] and high placental infection rates [162].

Such IPTp treated mothers contain high parasite diversity, increased level of parasitemia and severe inflammation in the placenta. It all happens due to changes in allele frequency at DHPS codon 581 in *Plasmodium falciparum* during pregnancy. Hence, regular assessment and chemoprophylaxis of malaria during pregnancy is highly needful to know the severity of infection caused by *Plasmodium falciparum* and physiological adverse effects imposed by the antibiotics on mother and her fetus [163]. For this purpose, routine screening of *P. falciparum* infection must be done up to delivery [164]. Drug susceptibility can be predicted by determining the IC50 values of drug in vitro studies being used. Increased IC50 of a drug determined in clinical isolates represent an instant increase in number of mutations occurred in the malaria parasite, but it is not absolutely true, because sometimes a single mutation occurred in any isolate may responsible for treatment failure in case of a particular drug but do not against all drugs. Undoubtedly, if two or three mutations observed in clinical isolates have shown very high drug resistance level [165]. Meanwhile, few drugs like quinine, amodiquinine, chloroquine, pyronaridine and sulfadoxine/pyrimethamine have shown very high ID50 values i.e. 46, 480, 52, 150, 15, and 10(4) nmol/L [166] *in vitro* against *P. falciparum*, hence rejected from use only after to confirmation of mutations and treatment failures occurred [167]. Hence, most of the drugs such as sulfadoxine-pyrimethamine (SP) have shown high IC_{50} values against *P. falciparum* and are totally banned in Africa [168]. But, due to low IC_{50} values Ciprofloxacin and norfloxacin are prescribed by physicianstp kill infections generated by *P. falciparum* [169].

Origin of Gametocytaemia

Long term use of non-effective drugs has increased transmission rate [170] of drug resistant falciparum parasite [171] and its infection rate in vector mosquitoes [172]. However, post treatment with CQ alone [173] or SP and CQ both [174] have shown a significant increase in the density of gametocytes [175] that resulted in gametocytaemia [176] in patients mainly in children [177]. It also reduces genotype formation in malaria infected patients [178] and enhances severity of pathogenesis [179]. To check the acute malaria cases patients should be monitored at an early stage of infection for their proper diagnosis and treatment to reduce the risk of disease progression and gametocyte carriage [180]. In such cases placental infection modulates the appearance of drug resistance in *P. falciparum* in pregnant women mainly in HIV positive women patients [181]. Contrary to this, few patients are able to clear genetically resistant *P. falciparum* genotype [182] that depends on adjustment of endogenous folate level, age, and resistance conferring mutations [183]. In conditions of acute infections, mosquito bites should be avoided [184] because from infected persons mosquitoes lifted gametocytes to generate sporozoites in sexual cycle and so they transmit it to new uninfected person. In endemic areas CQ-R resistant falciparum harbors in acute malaria patients which also work as large reservoirs of gametocytes. However, certain drugs like primaquine, artemisinin and its derivatives in combination are used to lower down gametocyte carriage parasite density, which reduces the chances of re-infection in treated individuals [185]. Hence, well tested treatment strategies are to be used for successful combating of the occurrence of gametocytaemia in patients [186].

Refugee camps are source of drug resistance alleles

Because of instability in eastern Afghanistan, new refugees crossed into the federally administered tribal areas of northwestern Pakistan in 2002. Investigators have identified an epidemic of *Plasmodium falciparum* malaria in 1 of the camps. Incidence was 100.4 cases/1,000 person-years; in other nearby camps it was only 2.1/1,000 person-years. Anopheline mosquitoes were found despite even after spray campaigns. The main clinical failure identified was used of locally manufactured sulfadoxine-pyrimethamine for routine treatment. *In vivo* failure rate was 28.5% and PCR analysis of the *P. falciparum* dihydrofolate reductase and dihyropteroate synthase genes showed no mutations associated with clinical failure. Therefore, clinically standard drug regimens should be used at global to level decrease incidence rate and rising malarial epidemics. To check this threat, enhanced quality assurance of control interventions is essential [44]. Molecular monitoring of parasite resistance is more important to launch anti-malarial drug policies. Again there is a possibility that large number of refugees surging up in European countries from Syria, Iraq, Lebanon and Afghanistan will sit in camps and acquire drug resistant malarial strains in future. Hence, testing of community samples for molecular drug resistance new bio- markers should be explored to be using them complementary tool for decision-making for the best treatment options and appropriate potential alternatives [187]. In addition, indicators based on molecular data have to be considered with caution and interpreted in the local context, especially with regard to prior drug usage and level of pre-existing immunity. For finding different climate and drug induced changes large scale genotyping and genetic mapping be needed in Plasmodium parasites. There must be identification of reasons of clinical failures due to selection and prescription of anti-malarial drugs. It is also important to identify effects of patient treatment on non-resistance group. Here, it is suggested that current scientific challenge regarding drug resistance should be accepted and all different reasons related to patient treatment regimens, prevention of pathogen transmission and developing mutations in malaria parasite will be explored to achieve complete elimination of drug resistant malaria as fast as possible.

Conclusion

In the present time control of multidrug resistant *P. falciparum* malaria has become a very difficult task because endogenous allelic exchanges occurred in *P. falciparum* have increased the therapeutic failures and significantly increased the levels of resistance worldwide. Big question here is how formation of drug resistant mutant alleles stops, because evolution is unending process. It is an important issue that has many dimensions to study and most important are demographic, eco-climatic and eco-genetic issues. Demographic issues are manmade while origin of malaria is natural and widely concerned to eco-climatic conditions. Hence, it is a great challenge, how to check the movements of symptomatic carriers that are responsible for transmission and dispersal among the non-infected human population across the continents. Further, genomic adaptations generated in *P. falciparum* in such carriers are proved highly prone to new mutations and many more genetic exchanges are possible when such migrants mix with uninfected population. Highly adapted and pesticide resistant local mosquito strains in endemic regions invited new possible mutations with in them and P. falciparum. Hence, there is a need to determine the factors related to adherence of various drugs and resistance grown in *Plasmodium falciparum*. However, for quick analysis of genomic polymorphism or diversity of natural parasite population exists in *P. falciparum* polymorphic microsatellite markers are to be used. With the help of this technology one can explore origin, pattern and spread of drug resistant *P. falciparum* worldwide. It may also help to find occurrence of new independent lineages and routes of geographical spread of resistance. Further, comparison of molecular evolutionary analyses of samples collected from various endemic regions can help to explore existence of multi-lineage drug resistance. Therefore there is a need to collect molecular epidemiological information regarding changes occurring at genomic level for avoiding the widespread distribution of high levels of drug resistant malaria parasite to stop it from spreading among non-infected human population.

References

1. Menard D, Djalle D,Yapou F, Manirakiza A, Talarmin A (2006) Frequency distribution of antimalarial drug-resistant alleles among isolates of *Plasmodium falciparum* in Bangui, Central African Republic. *Am. J. Trop. Med. Hyg*. 74: 205-210.
2. Kassa M, Sileshi M, Mohammed H, Taye G, Asfaw M (2005) Development of resistance by *Plasmodium falciparum* to sulfadoxine/pyrimethamine in Amhara Region, Northwestern Ethiopia. *Ethiop Med J*43: 181-187.
3. Salako LA, Ajayi FO, Sowunmi A, Walker O (1990) Malaria in Nigeria: a revisit. *Ann Trop Med Parasitol* 84: 435-445.[Crossref]
4. Shah NK, Dhillon GP, Dash AP, Arora U, Meshnick SR, et al. (2011) Antimalarial drug resistance of *Plasmodium falciparum* in India: changes over time and space. *Lancet Infect Dis* 11: 57-64.[Crossref]
5. Le Bras J, Musset L, Clain J (2006) [Antimalarial drug resistance]. *Med Mal Infect* 36: 401-405.[Crossref]
6. Nsimba B, Jafari-Guemouri S, Malonga DA, Mouata AM, Kiori J, et al. (2005) Epidemiology of drug-resistant malaria in Republic of Congo: using molecular evidence for monitoring antimalarial drug resistance combined with assessment of antimalarial drug use. *Trop Med Int Health* 10: 1030-1037.
7. Robert V, Molez JF, Trape JF (1996) Gametocytes, chloroquine pressure, and the relative parasite survival advantage of resistant strains of falciparum malaria in West Africa. *Am J Trop Med Hyg* 55: 350-351.
8. Smithuis FM, Monti F, Grundl M, Oo AZ, Kyaw TT, et al. (1997) *Plasmodium falciparum*: sensitivity in vivo to chloroquine, pyrimethamine/sulfadoxine and mefloquine in western Myanmar. *TransR Soc Trop Med*91:468-472.
9. Plowe CV (2009) The evolution of drug-resistant malaria. *Trans R Soc Trop Med Hyg* 103 Suppl 1: S11-14.[Crossref]
10. Obonyo CO, Juma EA, Ogutu BR, Vulule JM, Lau J (2007) Amodiaquine combined with sulfadoxine/pyrimethamine versus artemisinin-based combinations for the treatment of uncomplicated falciparum malaria in Africa: a meta-analysis. *Trans R Soc Trop Med Hyg 101*: 117-126.
11. Wallace MR, Sharp TW, Smoak B, Iriye C, Rozmajzl P, et al. (1996) Malaria among United States troops in Somalia. *Am J Med* 100: 49-55.[Crossref]
12. Woitsch B, Wernsdorfer G, Prajakwong S, Rojanawatsirivet C, Kollaritsch H, et al. (2004) Comparative study of the in vitro sensitivity of *Plasmodium falciparum* to artemisinin in two border areas of Thailand. *Wien Klin Wochenschr* 116 Suppl 4: 35-40. [Crossref]
13. Enosse S, Magnussen P, Abacassamo F, Gómez-Olivé X, Rønn AM, et al. (2008) Rapid increase of *Plasmodium falciparum* dhfr/dhps resistant haplotypes, after the adoption of sulphadoxine-pyrimethamine as first line treatment in 2002, in southern Mozambique. *Malar J* 7: 115.
14. Karema C, Imwong M, Fanello CI, Stepniewska K, Uwimana A, et al. (2010) Molecular correlates of high-level antifolate resistance in Rwandan children with *Plasmodium falciparum* malaria. *Antimicrob Agents Chemother* 54: 477-483.
15. Dlamini SV, Beshir K, Sutherland CJ(2010) Markers of anti-malarial drug resistance in *Plasmodium falciparum* isolates from Swaziland: identification of pfmdr1-86F in natural parasite isolates. *Malar J* 9: 68.
16. Ballif M, Hii J,Marfurt J, Crameri A,Fafale A, et al. (2010) Monitoring of malaria parasite resistance to chloroquine and sulphadoxine-pyrimethamine in the Solomon Islands by DNA microarray technology. *Malar J* 9: 270.
17. Zakeri S, Farahani MS, Afsharpad M, Salehi M, Raeisi A, et al. (2010) High prevalence of the 437G mutation associated with sulfadoxine resistance among *Plasmodium falciparum* clinical isolates from Iran, three years after the introduction of sulfadoxine-pyrimethamine. *Int J Infect Dis* 14: 123-128.
18. Ogunfowokan O, Dankyau M, Madaki AJ, Thacher TD (2009) Short report: comparison of chlorproguanil-dapsone with a combination of sulfadoxine-pyrimethamine and chloroquine in children with malaria in northcentral Nigeria. *Am J Trop Med Hyg* 80: 199-201.[Crossref]
19. Spalding MD, Eyase FL, Akala HM, Bendo SA, Prigge ST, et al. (2010) Increased prevalence of the pfdhfr/phdhps quintuple mutant and rapid emergence of pfdhps resistance mutation at codons 581 and 613 in Kisumu, Kenya. *Malar J* 9: 338.
20. Gasasira AF, Kamya MR, Ochong EO, Vora N, Achan J, et al. (2010) Effect of trimethoprim-sulphamethoxazole on the risk of malaria in HIV-infected Ugandan children living in an area of widespread antifolate resistance. *Malar J* 9: 177.
21. Strömberg A, Björkman A (1992) Ciprofloxacin does not achieve radical cure of *Plasmodium falciparum* infection in Sierra Leone. *Trans R Soc Trop Med Hyg* 86: 373. [Crossref]
22. Caraballo A, Rodriguez-Acosta A (1999) Chemotherapy of malaria and resistance to antimalarial drugs in Guayana area, Venezuela. *Am J Trop Med Hyg* 61: 120-124. [Crossref]
23. Salako LA, Ajayi FO, Sowunmi A, Walker O (1990) Malaria in Nigeria: a revisit. *Ann Trop Med Parasitol* 84: 435-445.[Crossref]
24. Mulenga, M., F. Malunga, S. Bennett, P.E. Thuma, C. Shulman, K. Fielding, A. Allioueche, and B.M. Greenwood. 2006a A randomised, double-blind, placebo-controlled trial of atovaquone-proguanil vs. sulphadoxine-pyrimethamine in the treatment of malarial anaemia in Zambian children. *Trop Med Int Health* 11: 1643-1652.
25. Bustos DG, Canfield CJ, Canete-Miguel E, Hutchinson BD(1999) Atovaquone-proguanil compared with chloroquine and chloroquine-sulfadoxine-pyrimethamine for treatment of acute *Plasmodium falciparum* malaria in the Philippines. *J Infect Dis* 179: 1587-1590.
26. Mulenga M, VangGeertruyden JP, Mwananyanda L, Chalwe V, Moerman F, et al.

(2006b) Safety and efficacy of lumefantrine-artemether (Coartem) for the treatment of uncomplicated *Plasmodium falciparum* malaria in Zambian adults. *Malar J* 5: 73.

27. Alam MT, Vinayak S, Congpuong K, Wongsrichanalai C, Satimai W, et al. (2011) Tracking origins and spread of sulfadoxine-resistant *Plasmodium falciparum* dhps alleles in Thailand. *Antimicrob Agents Chemother* 55: 155-164.

28. Alker AP, Lim P, Sem R, Shah NK, Yi P, et al. (2007) PFMDR1 and in vivo resistance to artesunate-mefloquine in falciparum malaria on the Cambodian-Thai border. *Am J Trop Med Hyg* 76:641-647.

29. Bonizzoni M, Afrane Y, Baliraine FN, Amenya DA, Githeko AK, et al. (2009) Genetic structure of *Plasmodium falciparum* populations between lowland and highland sites and antimalarial drug resistance in Western Kenya. *Infect Genet Evol* 9: 806-812.

30. Miao M, Yang Z, Cui L, Ahlum J, Huang Y, et al. (2010) Different allele prevalence in the dihydrofolate reductase and dihydropteroate synthase genes in *Plasmodium vivax* populations from China. *Am J Trop Med Hyg* 83: 1206-1211.[Crossref]

31. Mbacham WF, Evehe MS, Netongo PM, Ateh IA, Mimche PN, et al. (2010) Efficacy of amodiaquine, sulphadoxine-pyrimethamine and their combination for the treatment of uncomplicated *Plasmodium falciparum* malaria in children in Cameroon at the time of policy change to artemisinin-based combination therapy. *Malar J* 9: 34.

32. Hagos B, Khan B, Ofulla AV, Kariuki D, Martin SK (1993) Response of falciparum malaria to chloroquine and three second line antimalarial drugs in a Kenyan coastal school age population. *East Afr Med J* 70: 620-623.[Crossref]

33. Ekanem OJ, Weisfeld JS, Salako LA, Nahlen BL, Ezedinachi EN, et al. (1990) Sensitivity of *Plasmodium falciparum* to chloroquine and sulfadoxine/pyrimethamine in Nigerian children. *Bull World Health Organ* 68: 45-52.[Crossref]

34. Ogungbamigbe TO, Ojurongbe O, Ogunro PS, Okanlawon BM, Kolawole SO(2008) Chloroquine resistant *Plasmodium falciparum* malaria in Osogbo Nigeria: efficacy of amodiaquine + sulfadoxine-pyrimethamine and chloroquine + chlorpheniramine for treatment. *Mem Inst Oswaldo Cruz* 103: 79-84.

35. Vicente D, Pérez-Trallero E (2010) [Tetracyclines, sulfonamides, and metronidazole]. *Enferm Infecc Microbiol Clin* 28: 122-130.[Crossref]

36. Thanh NV, Toan TQ, Cowman AF, Casey GJ, Phuc BQ, et al. (2010) Monitoring for *Plasmodium falciparum* drug resistance to artemisinin and artesunate in Binh Phuoc Province, Vietnam: 1998-2009. *Malar J* 9: 181.[Crossref]

37. Whegang SY, Tahar R, Foumane VN, Soula G, Gwét H, et al. (2010) Efficacy of non-artemisinin- and artemisinin-based combination therapies for uncomplicated falciparum malaria in Cameroon. *Malar J* 9: 56.

38. Okafor HU, Shu EN, Oguonu T(2010) Therapeutic efficacy and effect on gametocyte carriage of an artemisinin and a non-based combination treatment in children with uncomplicated *P. falciparum* malaria, living in an area with high-level chloroquine resistance. *J Trop Pediatr* 56: 398-406.

39. Basco LK, Ngane M, Ndounga A, Same-EkoboJC, YoumbaRT, et al. (2006) Molecular epidemiology of malaria in Cameroon. XXI. Baseline therapeutic efficacy of chloroquine, amodiaquine, and sulfadoxine-pyrimethamine monotherapies in children before national drug policy change. *Am J Trop Med Hyg* 75: 388-395.

40. Nzila AM, Kokwaro G, Winstanley PA, Marsh K, Ward SA (2004) Therapeutic potential of folate uptake inhibition in *Plasmodium falciparum*. *Trends Parasitol* 20: 109-112.[Crossref]

41. de Beer TA, LouwAI, Joubert F(2006) Elucidation of sulfadoxine resistance with structural models of the bifunctional *Plasmodium falciparum* dihydropterin pyrophosphokinase-dihydropteroate synthase. *Bioorg Med Chem* 14: 4433-4443.

42. Bukirwa H, Critchley J (2006) Sulfadoxine-pyrimethamine plus artesunate versus sulfadoxine-pyrimethamine plus amodiaquine for treating uncomplicated malaria. *Cochrane Database Syst Rev* 25: 4966-4970.

43. Marks F, von Kalckreuth V, Kobbe R, Adjei S, Adjei O, et al. (2005) Parasitological rebound effect and emergence of pyrimethamine resistance in *Plasmodium falciparum* after single-dose sulfadoxine-pyrimethamine. *J Infect Dis* 192:1962-1965.

44. Leslie T, Mayan MI, Hasan MA, Safi MH, Klinkenberg E, et al. (2007) Sulfadoxine-pyrimethamine, chlorproguanil-dapsone, or chloroquine for the treatment of Plasmodium vivax malaria in Afghanistan and Pakistan: a randomized controlled trial. *JAMA* 297: 2201-2209.[Crossref]

45. Bousema JT, Gouagna LC, Drakeley CJ, Meutstege AM, Okech BA, et al. (2004) *Plasmodium falciparum* gametocyte carriage in asymptomatic children in western Kenya. *Malar J* 3: 18.[Crossref]

46. Khatoon L, Baliraine FN, Bonizzoni M, Malik SA, Yan G (2009) Prevalence of antimalarial drug resistance mutations in *Plasmodium vivax* and *P. falciparum* from a malaria-endemic area of Pakistan. *Am J Trop Med Hyg* 81: 525-528.[Crossref]

47. Sharma VP (1996) Re-emergence of malaria in India. *Indian J Med Res* 103: 26-45.[Crossref]

48. Ghosh SK, Yadav RS, Sharma VP (1992) Sensitivity status of *Plasmodium falciparum* to chloroquine, amodiaquine, quinine, mefloquine and sulfadoxine/pyrimethamine in a tribal population of District Sundargarh, Orissa. Indian. *J Malariol* 29: 211-218.

49. Dua VK, Kar PK, Sharma VP (1996) Chloroquine resistant Plasmodium vivax malaria in India. *Trop Med Int Health* 1: 816-819.[Crossref]

50. Sharma YD, Biswas S, Pillai CR, Ansari MA, Adak T, et al. (1996) High prevalence of chloroquine resistant *Plasmodium falciparum* infection in Rajasthan epidemic. *Acta Trop* 62: 135-141.[Crossref]

51. Dua VK, Nanda N, Gupta NC, Kar PK, Subbarao SK, et al. (2000) Investigation of malaria prevalence at National Thermal Power Corporation, Shaktinagar, Sonbhadra District (Uttar Pradesh), India. *Southeast Asian J Trop Med Public Health* 31: 818-824.

52. Souares A, Lalou R, Sene I, Sow D, Le Hesran JP(2006) Knowledge and practice among health workers from the Thiès region with regard to new malaria treatment policies. *Sante Publique* 18: 299-310.

53. McCollum AM, Poe AC, Hamel M, Huber C, Zhou Z, et al. (2006) Antifolate resistance in *Plasmodium falciparum*: multiple origins and identification of novel dhfr alleles. *J Infect Dis* 194: 189-197.[Crossref]

54. Gatton ML, Cheng Q(2006) *Plasmodium falciparum* infection dynamics and transmission potential following treatment with sulfadoxine-pyrimethamine. *J Antimicrob Chemother* 58: 47-51.

55. Uhlemann AC, Ramharter M, Lell B, Kremsner PG, Krishna S (2005) Amplification of *Plasmodium falciparum* multidrug resistance gene 1 in isolates from Gabon. *J Infect Dis* 192: 1830-1835.[Crossref]

56. Ayala E, Lescano AG, Gilman RH, Calderón M, Pinedo VV, et al. (2006) Polymerase chain reaction and molecular genotyping to monitor parasitological response to anti-malarial chemotherapy in the Peruvian Amazon. *Am J Trop MedHyg* 74: 546-553.

57. A-Elbasit IE, Khalil IF, Elbashir MI, Masuadi EM, Bygbjerg IC, et al. (2008) High frequency of *Plasmodium falciparum* CICNI/SGEAA and CVIET haplotypes without association with resistance to sulfadoxine/pyrimethamine and chloroquine combination in the Daraweesh area, in Sudan. *Eur J Clin Microbiol Infect Dis* 27:725-732.

58. Hamel MJ, Poe A, Bloland P,McCollum A, Zhou Z, et al. (1999) Dihydrofolate reductase I164L mutations in *Plasmodium falciparum* isolates: clinical outcome of 14 Kenyan adults infected with parasites harbouring the I164L mutation. *Trans R Soc Trop Med Hyg* 102: 338-345.

59. Sokhna C, Cissé B, Bâel H, Milligan P, Hallett R, et al. (2008) A trial of the efficacy, safety and impact on drug resistance of four drug regimens for seasonal intermittent preventive treatment for malaria in Senegalese children. *PLoS One* 3: 1471.

60. Vinayak S, Alam MT,Upadhyay M, Das MK, Dev V, et al. (2007) Extensive genetic diversity in the *Plasmodium falciparum* Na+/H+ exchanger 1 transporter protein implicated in quinine resistance. *Antimicrob Agents Chemother* 51: 4508-4511.

61. Méndez F, Herrera S, Murrain B, Gutiérrez A, Moreno LA, et al. (2001) Selection of antifolate-resistant *Plasmodium falciparum* by sulfadoxine-pyrimethamine treatment and infectivity to Anopheles mosquitoes. *Am J Trop Med Hyg* 77: 438-443.

62. Enevold A (2006) The influence of genetic innate resistance and acquired immunity on drug treatment outcome of uncomplicated *Plasmodium falciparum* malaria in Tanzania. *Parassitologia* 48: 547-551.[Crossref]

63. Mayxay M, Nair S, Sudimack D, Imwong M, Tanomsing N, et al. (2007) Combined molecular and clinical assessment of *Plasmodium falciparum* antimalarial drug resistance in the Lao People's Democratic Republic (Laos). *Am J Trop Med Hyg* 77: 36-43.

64. Akeri S, Afsharpad M, Raeisi A, Djadid ND(2007) Prevalence of mutations associated with antimalarial drugs in *Plasmodium falciparum* isolates prior to the introduction of sulphadoxine-pyrimethamine as first-line treatment in Iran. *Malar J* 6: 148.

65. Mita T, Kaneko A, Hwaihwanje I, Tsukahara T, Takahashi N, et al. (2006) Rapid selection of dhfr mutant allele in *Plasmodium falciparum* isolates after the introduction of sulfadoxine/pyrimethamine in combination with 4-aminoquinolines in Papua New Guinea. *Infect Genet Evol* 6: 447-452.

66. Fernandes N, Figueiredo P, do VE Rosário, Cravo P (2007) Analysis of sulphadoxine/pyrimethamine resistance-conferring mutations of *Plasmodium falciparum* from Mozambique reveals the absence of the dihydrofolate reductase 164L mutant. *Malar J* 6: 35.

67. Al Harthi SA (2007) Detection of drug resistance markers for chloroquine and pyrimethamine-sulfadoxine in Jazan area, Saudi Arabia using PCR and restriction digestion. *J Egypt Soc Parasitol* 37: 17-30.[Crossref]

68. Durand S, Marquiño W, Cabezas C, Utz G, Fiestas V, et al. (2007) Unusual pattern of *Plasmodium falciparum* drug resistance in the northwestern Peruvian Amazon region. *Am J Trop Med Hyg* 76: 614-618.[Crossref]

69. Tinto H, Ouédraogo JB, Zongo I, van Overmeir C, van Marck E, et al. (2007) Sulfadoxine-pyrimethamine efficacy and selection of *Plasmodium falciparum* DHFR mutations in Burkina Faso before its introduction as intermittent preventive treatment for pregnant women. *Am J Trop Med Hyg* 76: 608-613.

70. Schousboe ML, Rajakaruna RS, Salanti A, Hapuarachchi HC, Galappaththy GN, et al. (2007) Island-wide diversity in single nucleotide polymorphisms of the *Plasmodium vivax* dihydrofolate reductase and dihydropteroate synthetase genes in Sri Lanka. *Malar J* 6: 28.

71. Djaman JA, Mazabraud A, Basco L(2007) Sulfadoxine-pyrimethamine susceptibilities and analysis of the dihydrofolate reductase and dihydropteroate synthase of *Plasmodium falciparum* isolates from Côte d'Ivoire. *Ann Trop Med Parasitol* 101: 103-112.

72. Mugittu K, Priotto G, Guthmann JP, Kiguli J, Adjuik M, et al. (2007) Molecular genotyping in a malaria treatment trial in Uganda - unexpected high rate of new infections within 2 weeks after treatment. *Trop Med Int Health* 12: 219-223.

73. Diallo DA, Sutherland C, Nebié I, Konaté AT, Ord R, et al. (2007) Sustained use of insecticide-treated curtains is not associated with greater circulation of drug-resistant malaria parasites, or with higher risk of treatment failure among children with uncomplicated malaria in Burkina Faso. *Am J Trop Med Hyg* 76: 237-244.[Crossref]

74. Sowunmi A, Adedeji AA, Gbotosho GO, Fateye BA, Happi TC(2006) Effects of pyrimethamine-sulphadoxine, chloroquine plus chlorpheniramine, and amodiaquine plus pyrimethamine-sulphadoxine on gametocytes during and after treatment of acute, uncomplicated malaria in children. *Mem Inst Oswaldo Cruz* 101: 887-893.

75. Morrow RH (2007) Antimalarial drug combinations in vastly different settings. *Lancet* 369: 444-445.[Crossref]

76. McCollum AM, Mueller K, Villegas L, Udhayakumar V, Escalante AA(2007) Common origin and fixation of *Plasmodium falciparum* dhfr and dhps mutations associated with sulfadoxine-pyrimethamine resistance in a low-transmission area in South America. Antimicrob. *Agents Chemother* 51: 2085-2091.

77. Crawley J, Hill J, Yartey J, Robalo M, Serufilira A, et al. (2007) From evidence to action? Challenges to policy change and programme delivery for malaria in pregnancy. *Lancet Infect Dis* 7: 145-155.[Crossref]

78. Schönfeld M, Barreto Miranda I, Schunk M, Maduhu I, Maboko L, et al. (2007) Molecular surveillance of drug-resistance associated mutations of *Plasmodium falciparum* in south-west Tanzania. *Malar J* 6: 2.

79. Genton B, Baea K, Lorry K, Ginny M, Wines B, et al. (2005) Parasitological and clinical efficacy of standard treatment regimens against *Plasmodium falciparum*, *P. vivax* and P. malariae in Papua New Guinea. *P N G Med J* 48: 141-150.

80. Noranate NR, Durand A, Tall L, Marrama A, Spiegel C, et al. (2007) Rapid dissemination of *Plasmodium falciparum* drug resistance despite strictly controlled antimalarial use. *PLoS One*2: 139.

81. Kofoed PE, Rodrigues A, Aaby P, Rombo L (2006) Continued efficacy of sulfadoxine-pyrimethamine as second line treatment for malaria in children in Guinea-Bissau. *Acta Trop* 100: 213-217.[Crossref]

82. Carnevale EP, Kouri D, DaRe JT, McNamara DT, Mueller I, et al. (2007) A multiplex ligase detection reaction-fluorescent microsphere assay for simultaneous detection of single nucleotide polymorphisms associated with *Plasmodium falciparum* drug resistance. *J Clin Microbio*l 45: 752-761.

83. Laufer MK, Thesing PC, Eddington ND, Masonga R, Dzinjalamala FK, et al. (2006) Return of chloroquine antimalarial efficacy in Malawi. *N Engl J Med* 355: 1959-1966. [Crossref]

84. Mockenhaupt FP, Bedu-Addo G, Junge C, Hommerich L, Eggelte TA, et al. (2006) Markers of sulfadoxine-pyrimethamine-resistant *Plasmodium falciparum* in placenta and circulation of pregnant women. *Antimicrob Agents Chemother* 51: 332-334.

85. Mbugi EV, Mutayoba BM, Malisa AL, Balthazary ST, Nyambo TB, et al. (2006) Drug resistance to sulphadoxine-pyrimethamine in *Plasmodium falciparum* malaria in Mlimba, Tanzania. *Malar J* 5: 94.[Crossref]

86. Mulenga M, Malunga F, Bennett S, Thuma PE, Shulman C, et al. (2006) A randomised, double-blind, placebo-controlled trial of atovaquone-proguanil vs. sulphadoxine-pyrimethamine in the treatment of malarial anaemia in Zambian children. *Trop Med Int Health* 11: 1643-1652.

87. Tagbor H, Bruce J, Browne E, Randal A, Greenwood B, et al. (2006) Efficacy, safety, and tolerability of amodiaquine plus sulphadoxine-pyrimethamine used alone or in combination for malaria treatment in pregnancy: a randomised trial. *Lancet* 368: 1349-1356.[Crossref]

88. Thriemer K, Haque R, Wagatsuma Y, Salam MA, Akther S, et al. (2006) Therapeutic efficacy of quinine plus sulfadoxine-pyremethamine for the treatment of uncomplicated falciparum malaria in Bangladesh. *Am J Trop Med Hyg* 75: 645-649.

89. Méndez F, Muñoz A,Plowe CV (2005) Use of area under the curve to characterize transmission potential after antimalarial treatment. *Am J Trop Med Hyg* 75: 640-644.

90. Auliff A, Wilson DW, Russell B, Gao Q, Chen N, et al. (2006) Amino acid mutations in Plasmodium vivax DHFR and DHPS from several geographical regions and susceptibility to antifolate drugs. *Am J Trop Med Hyg* 75: 617-621.

91. Ratcliff A, Siswantoro H, Kenangalem E, Wuwung M, Brockman A, et al. (2006) Therapeutic response of multidrug-resistant *Plasmodium falciparum* and *P. vivax* to chloroquine and sulfadoxine-pyrimethamine in southern Papua, Indonesia. *Trans R Soc Trop Med Hyg* 101: 351-359.

92. Fanello CI, Karema C, Van W, Doren C, Van O, et al. (2006) A randomised trial to assess the safety and efficacy of artemether-lumefantrine (Coartem) for the treatment of uncomplicated *Plasmodium falciparum* malaria in Rwanda. *Trans R Soc Trop Med Hyg* 101: 344-350.

93. Swarthout TD, van den Broek IV, Kayembe G, Montgomery J, Pota H, et al. (2008) Artesunate + amodiaquine and artesunate + sulphadoxine-pyrimethamine for treatment of uncomplicated malaria in Democratic Republic of Congo: a clinical trial with determination of sulphadoxine and pyrimethamine-resistant haplotypes. *Trop Med Int Health* 11:1503-1511.

94. Tinto H, Sanou B, Erhart A, D'Alessandro U, Ouédraogo JB, et al. (1998) Guiguemdé. In vivo sensitivity of Plasmodium faciparum to chloroquine and sulfadoxine pyrimethamine in the Bobo Dioulasso region (1998-2001): risk factors associated with treatments failures to the two drugs. *Bull Soc Pathol Exot* 99: 161-165.

95. Ali E, Mackinnon MJ, Abdel-Muhsin AM, Ahmed S, Walliker D, et al. (2006) Increased density but not prevalence of gametocytes following drug treatment of *Plasmodium falciparum*. *Trans R Soc Trop Med Hyg* 100: 176-183.[Crossref]

96. Dokomajilar C, Lankoande ZM, Dorsey G, Zongo I, Ouedraogo JB, et al. (2006) Roles of specific *Plasmodium falciparum* mutations in resistance to amodiaquine and sulfadoxine-pyrimethamine in Burkina Faso. *Am J Trop Med Hyg* 75: 162-165. [Crossref]

97. Cohuet S, Bonnet M, Van Herp M, Van Overmeir C, D'Alessandro U, et al. (2006) Short report: molecular markers associated with *Plasmodium falciparum* resistance to sulfadoxine-pyrimethamine in the Democratic Republic of Congo. *Am J Trop Med Hyg* 75: 152-154.

98. Schunk M, Kumma WP, Miranda IB, Osman ME, Roewer S, et al. (2006) High prevalence of drug-resistance mutations in *Plasmodium falciparum* and *Plasmodium vivax* in southern Ethiopia. *Malar J* 5: 54.[Crossref]

99. Platteeuw JJ (2006) Resistance to sulphadrug-based antifolate therapy in malaria: are we looking in the right place? *Trop Med Int Health* 11: 804-808.[Crossref]

100. Mbaye A, Richardson K, Balajo B, Dunyo S, Shulman C, et al. (2006) Lack of inhibition of the anti-malarial action of sulfadoxine-pyrimethamine by folic acid supplementation when used for intermittent preventive treatment in Gambian primigravidae. *Am J Trop Med Hyg* 74: 960-964.

101. Valecha N, Joshi H, Eapen A, Ravinderan J, Kumar A, et al. (2006) Therapeutic efficacy of chloroquine in Plasmodium vivax from areas with different epidemiological patterns in India and their Pvdhfr gene mutation pattern. *Trans R Soc Trop Med Hyg* 100: 831-837.

102. Francis D, Nsobya SL, Talisuna A, Yeka A, Kamya MR, et al. (2006) Geographic differences in antimalarial drug efficacy in Uganda are explained by differences in endemicity and not by known molecular markers of drug resistance. *J Infect Dis* 193: 978-986.[Crossref]

103. Tjitra E, S. Suprianto, NM Anstey (2002) Higher gametocyte prevalence following failure of treatment of *Plasmodium falciparum* malaria with sulfadoxine–pyrimethamine and the combination of chloroquine plus sulfadoxine-pyrimethamine: implications for progression of anti-folate resistance. *Trans Royal Soc Trop Med Hyg* 96: 434-437.

104. Rodenko B, RJ Detz, VA Pinas, C Lambertucci, R Brun, et al. (2006)

Solid phase synthesis and antiprotozoal evaluation of di- and trisubstituted 5'-carboxamidoadenosine analogues. *Bioorg Med Chem* 14: 1618-1629.

105. Mubyazi GM, Gonzalez-Block MA (2005) Research influence on antimalarial drug policy change in Tanzania: case study of replacing chloroquine with sulfadoxine-pyrimethamine as the first-line drug. *Malar J* 4: 51.[Crossref]

106. McIntosh HM, Jones KL (2005) Chloroquine or amodiaquine combined with sulfadoxine-pyrimethamine for treating uncomplicated malaria. *Cochrane Database Syst Rev* : CD000386.[Crossref]

107. Thera MA, Sehdev PS, Coulibaly D, Traore K, Garba MN, et al. (2005) Impact of trimethoprim-sulfamethoxazole prophylaxis on falciparum malaria infection and disease. *J Infect Dis* 192: 1823-1829.

108. Lemnge MM, Ali AS, Malecela EK, Sambu E, Abdulla R, et al. (1998) Therapeutic efficacy of sulfadoxine-pyrimethamine and amodiaquine among children with uncomplicated *Plasmodium falciparum* malaria in Zanzibar, Tanzania. *Am J Trop Med Hyg* 73: 681-685.

109. Wilson PE, Kazadi W, Alker AP, Meshnick SR (2005) Rare Congolese *Plasmodium falciparum* DHFR alleles. *Mol Biochem Parasitol* 144: 227-229.[Crossref]

110. Nyunt M, Pisciotta J, Feldman AB, Thuma P, Scholl PF, et al. (2005) Detection of *Plasmodium falciparum* in pregnancy by laser desorption mass spectrometry. *Am J Trop Med Hyg* 73: 485-490.[Crossref]

111. Mockenhaupt FP, Teun Bousema J, Eggelte TA, Schreiber J, Ehrhardt S, et al. (2005) *Plasmodium falciparum* dhfr but not dhps mutations associated with sulphadoxine-pyrimethamine treatment failure and gametocyte carriage in northern Ghana. *Trop Med Int Health* 10: 901-908.

112. Alker AP, Mwapasa V, Purfield A, Rogerson SJ, Molyneux ME, et al. (2005) Mutations associated with sulfadoxine-pyrimethamine and chlorproguanil resistance in *Plasmodium falciparum* isolates from Blantyre, Malawi. Antimicrob. *Agents Chemother* 49: 3919-3921.

113. Toma H, Imada Y, Vannachone B, Miyagi M, Kobayashi J, et al. (2005)A molecular epidemiologic study of point mutations for pyrimethamine-sulfadoxine resistance of *Plasmodium falciparum* isolates from Lao PDR. Southeast Asian.*J Trop Med Public Health* 36: 602-604.

114. Baruah I, Talukdar PK, Das SC (2005) The drug sensitivities of *Plasmodium falciparum* in the Sonitpur district, Assam, India. *Southeast Asian J Trop Med Public Health* 36: 587-590.[Crossref]

115. Abuaku BK, Koram KA, Binka FN (2005) Antimalarial prescribing practices: a challenge to malaria control in Ghana. *Med Princ Pract* 14: 332-337.[Crossref]

116. Eichner M, Diebner H, Molineaux L, Collins WE, Jeffery GM, et al. (2001) Genesis, sequestration and survival of *Plasmodium falciparum* gametocytes: parameter estimates from fitting a model to malariotherapy data. *Trans Royal Soc Trop Med Hyg* 95: 497-501.

117. Falade CO, Salako LA, Sowunmi A, Oduola AMJ, Larcier P (1997) Comparative efficacy of halofantrine, chloroquine and sulphadoxine–pyrimethamine in the treatment of acute uncomplicated falciparum malaria in Nigerian children. *Trans Royal Soc Trop Med Hyg* 91: 58-62.

118. Handunnetti SM, Gunewardena DM, Pathirana PPSL, Ekanayake K, Weerasinghe S & Mendis KN (1996) Features of recrudescent chloroquine-resistant *Plasmodium falciparum* infections confer a survival advantage on parasites and have implications for disease control. Trans. Royal Soc. Trop. Med. Hyg. 90: 563–567.

119. Nour BY, Faragalla IA, Saeed OK, Mohamadani AA (2006) In vitro study assessing the response of *Plasmodium falciparum* malaria to chloroquine, sulfadoxine/pyrimethamine, quinine and mefloquine in Wad Medani District, Sudan. *Saudi Med J* 27: 808-812.

120. Osman ME, Mockenhaupt FP, Bienzle U, Elbashir MI, Giha HA (2007) Field-based evidence for linkage of mutations associated with chloroquine (pfcrt/pfmdr1) and sulfadoxine-pyrimethamine (pfdhfr/pfdhps) resistance and for the fitness cost of multiple mutations in P. falciparum. *Infect Genet Evol* 7: 52-59.

121. Myrick A, Leemann E, Dokomajilar C, Hopkins H, Dorsey G, et al. (2006) Short report: Dynamics of *Plasmodium falciparum* malaria after sub-optimal therapy in Uganda. *Am J Trop Med Hyg* 74: 758-761.[Crossref]

122. A-Elbasit IE, Elbashir MI, Khalil IF, Alifrangis IM, Giha A(2006) The efficacy of sulfadoxine-pyrimethamine alone and in combination with chloroquine for malaria treatment in rural Eastern Sudan: the interrelation between resistance, age and gametocytogenesis. *Trop Med Int Health* 11: 604-612.

123. Kalanda GC, Hill J, Verhoeff FH, Brabin BJ (2006) Comparative efficacy of chloroquine and sulphadoxine--pyrimethamine in pregnant women and children: a meta-analysis. *Trop Med Int Health* 11: 569-577.[Crossref]

124. Hapuarachchi HC, Dayanath MY, Bandara KB, Abeysundara S, Abeyewickreme W, et al. (2006) Point mutations in the dihydrofolate reductase and dihydropteroate synthase genes of *Plasmodium falciparum* and resistance to sulfadoxine-pyrimethamine in Sri Lanka. *Am J Trop Med Hyg* 74: 198-204.

125. Jayatilaka KD, Taviri J, Kemiki A, Hwaihwanje I, Bulungol P (2003) Therapeutic efficacy of chloroquine or amodiaquine in combination with sulfadoxine-pyrimethamine for uncomplicated falciparum malaria in Papua New Guinea. *P N G Med J* 46: 125-134.[Crossref]

126. Mannoor MK, Vanisaveth V, Keokhamphavanh B, Toma H, Watanabe H, et al. (2005) Pyrimethamine-sulfadoxine treatment of uncomplicated *Plasmodium falciparum* malaria in Lao PDR. *Southeast Asian J Trop Med Public Health* 36: 1092-1095.

127. Gebru-Woldearegai T, Hailu A, Grobusch MP, Kun JF (2005) Molecular surveillance of mutations in dihydrofolate reductase and dihydropteroate synthase genes of *Plasmodium falciparum* in Ethiopia. *Am J Trop Med Hyg* 73: 1131-1134.[Crossref]

128. Grandesso F, Bachy C, Donam I, Ntambi J, Habimana J, et al. (2006) Efficacy of chloroquine, sulfadoxine-pyrimethamine and amodiaquine for treatment of uncomplicated *Plasmodium falciparum* malaria among children under five in Bongor and Koumra.*Chad Trans R Soc Trop Med Hyg* 100: 419-426..

129. Mugittu K, Abdulla S, Falk N, Masanja H, Felger I, et al. (2006) Efficacy of sulfadoxine-pyrimethamine in Tanzania after two years as first-line drug for uncomplicated malaria: assessment protocol and implication for treatment policy strategies. *Malar J* 4: 55.

130. Adjetey TA, Affoumou GB, Loukou DD, Nebavi NG, Barro-Kiki P, et al. (2005) [Evaluation of the therapeutic efficacy of amodiaquine versus chloroquine in the treatment of uncomplicated malaria in Abie, Côte-d'Ivoire]. *Bull Soc Pathol Exot* 98: 193-196.[Crossref]

131. Oesterholt MJ, Alifrangis M, Sutherland CJ, Omar SA, Sawa P, et al. (2009) Submicroscopic gametocytes and the transmission of antifolate-resistant *Plasmodium falciparum* in Western Kenya. PLoS One. 4(2): 4364.

132. Barnes KI, Little F, Mabuza A, Mngomezulu N, Govere J, et al. (2008) Increased gametocytemia after treatment: an early parasitological indicator of emerging sulfadoxine-pyrimethamine resistance in falciparum malaria. *J Infect Dis* 197:1605-1613.

133. Kerketta AS, Mohapatra SS, Kar SK(2008) Assessment of the therapeutic efficacy of chloroquine in the treatment of uncomplicated *Plasmodium falciparum* malaria in a tribal block of the Kalahandi district of Orissa, India. *Trop Doct* 38: 82-84.

134. Marfurt J, Müller I, Sie A, Oa O, Reeder JC, et al. (2008) The usefulness of twenty-four molecular markers in predicting treatment outcome with combination therapy of amodiaquine plus sulphadoxine-pyrimethamine against falciparum malaria in Papua New Guinea. *Malar J* 7: 61.

135. Mayor A, Serra-Casas E, Sanz S, Aponte JJ, Macete E, et al. (2008) Molecular markers of resistance to sulfadoxine-pyrimethamine during intermittent preventive treatment for malaria in Mozambican infants. *J Infect Dis* 197: 1737-1742.

136. Ogungbamigbe TO, Ojurongbe O, Ogunro PS, Okanlawon BM, Kolawole SO(2008) Chloroquine resistant *Plasmodium falciparum* malaria in Osogbo Nigeria: efficacy of amodiaquine + sulfadoxine-pyrimethamine and chloroquine + chlorpheniramine for treatment. *Mem Inst Oswaldo Cruz* 103: 79-84.

137. Djimdé AA, Fofana B, Sagara I, Sidibe B, Toure S, et al. (2008) Efficacy, safety, and selection of molecular markers of drug resistance by two ACTs in Mali. *Am J Trop Med Hyg* 78: 455-461.[Crossref]

138. Bell DJ, Nyirongo SK, Mukaka M, Zijlstra EE, Plowe CV, et al. (2008) Sulfadoxine-pyrimethamine-based combinations for malaria: a randomised blinded trial to compare efficacy, safety and selection of resistance in Malawi. *PLoS One* 3: 1578.

139. Nsimba B, Guiyedi V, Mabika-Mamfoumbi M, Mourou-Mbina JR, Ngoungou E, et al. (2008) Sulphadoxine/pyrimethamine versus amodiaquine for treating uncomplicated childhood malaria in Gabon: a randomized trial to guide national policy. *Malar J* 7: 31.

140. Eriksen J, Mwankusye S, Mduma S, Veiga MI, Kitua A, et al. (2008) Antimalarial resistance and DHFR/DHPS genotypes of *Plasmodium falciparum* three years after introduction of sulfadoxine-pyrimethamine and amodiaquine in rural Tanzania. *Trans R Soc Trop Med Hyg* 102: 137-142.

141. Kobbe R, Adjei S, Kreuzberg C, Kreuels B, Thompson B, et al. (2007) Malaria incidence and efficacy of intermittent preventive treatment in infants (IPTi). *Malar J* 6: 163.

142. Zhou Z, Griffing SM, de Oliveira AM, McCollum AM, Quezada WM, et al. (2008) Decline in sulfadoxine-pyrimethamine-resistant alleles after change in drug policy in the Amazon region of Peru. Antimicrob. *Agents Chemother* 52: 739-741.

143. Enevold A, Nkya WM, Theisen M, Vestergaard LS, Jensen AT, et al. (2007) Potential impact of host immunity on malaria treatment outcome in Tanzanian children infected with *Plasmodium falciparum*. *Malar J* 6: 153.

144. Marfurt J, Müeller I, Sie A, Maku P, Goroti M, et al. (2007) Low efficacy of amodiaquine or chloroquine plus sulfadoxine-pyrimethamine against *Plasmodium falciparum* and *P. vivax* malaria in Papua New Guinea. *Am J Trop Med Hyg* 77: 947-954.[Crossref]

145. Rulisa S, Gatarayiha JP, Kabarisa T, Ndayisaba G(2007) Comparison of different artemisinin-based combinations for the treatment of *Plasmodium falciparum* malaria in children in Kigali, Rwanda, an area of resistance to sulfadoxine-pyrimethamine: artesunate plus sulfadoxine/pyrimethamine versus artesunate plus sulfamethoxypyrazine/pyrimethamine. *Am J Trop Med Hyg* 77: 612-616.

146. Ndounga M, Tahar R, Basco LK, Casimiro PN, Malonga DA, et al. (2007) Therapeutic efficacy of sulfadoxine-pyrimethamine and the prevalence of molecular markers of resistance in under 5-year olds in Brazzaville, Congo. *Trop Med Int Health* 12: 1164-1171.[Crossref]

147. Fernandes NE, Cravo P,do Rosário VE(2007) Sulfadoxine-pyrimethamine resistance in Maputo, Mozambique: presence of mutations in the dhfr and dhps genes of *Plasmodium falciparum*. *Rev Soc Bras Med Trop* 40: 447-450.

148. Thapa S, Hollander J, Linehan M, Cox-Singh J, Bista MB, et al. (2007) Comparison of artemether-lumefantrine with sulfadoxine-pyrimethamine for the treatment of uncomplicated falciparum malaria in eastern Nepal. *Am J Trop Med Hyg* 77: 423-430.

149. Tahar R, Djaman J, Ferreira C, Basco L (2007) [Molecular surveillance of sulfadoxine-pyrimethamine-resistant *Plasmodium falciparum* in São Tomé and Príncipe]. *Bull Soc Pathol Exot* 100: 115-118.[Crossref]

150. Kolaczinski K, Durrani N, Rahim S(2007) Rowland. Sulfadoxine-pyrimethamine plus artesunate compared with chloroquine for the treatment of vivax malaria in areas co-endemic for *Plasmodium falciparum* and *P. vivax* : a randomised non-inferiority trial in eastern Afghanistan. *Trans R Soc Trop Med Hyg* 101: 1081-1087.

151. Tahar R, Basco LK(2007) Molecular epidemiology of malaria in Cameroon. XXVI. Twelve-year in vitro and molecular surveillance of pyrimethamine resistance and experimental studies to modulate pyrimethamine resistance. *Am J Trop Med Hyg* 77: 221-227.

152. A-Elbasit IE, Alifrangis M, Khalil IF, Bygbjerg IC, Masuadi EM, et al. (2001) The implication of dihydrofolate reductase and dihydropteroate synthetase gene mutations in modification of *Plasmodium falciparum* characteristics. *Malar J* 6: 108.

153. Faye B, Ndiaye JL, Ndiaye D, Dieng Y, Faye O, et al. (2007) Efficacy and tolerability of four antimalarial combinations in the treatment of uncomplicated *Plasmodium falciparum* malaria in Senegal. *Malar J* 6: 80.[Crossref]

154. Mlambo G, Sullivan D, Mutambu SL, Soko W, Mbedzi J, et al. (2007) High prevalence of molecular markers for resistance to chloroquine and pyrimethamine in *Plasmodium falciparum* from Zimbabwe. *Parasitol Res* 101: 1147-1151.[Crossref]

155. Nkhoma S, Molyneux M, Ward S (2007) Molecular surveillance for drug-resistant Plasmodiumfalciparum malaria in Malawi. *Acta Trop* 102: 138-142.[Crossref]

156. Peters PJ, Thigpen MC, Parise ME, Newman RD (2007) Safety and toxicity of sulfadoxine/pyrimethamine: implications for malaria prevention in pregnancy using intermittent preventive treatment. *Drug Saf* 30: 481-501.

157. Bonnet M, Roper C, Félix M, Coulibaly L, Kankolongo GM, et al. (2007) Efficacy of antimalarial treatment in Guinea: in vivo study of two artemisinin combination therapies in Dabola and molecular markers of resistance to sulphadoxine-pyrimethamine in N'Zérékoré. *Malar J* 6: 54.

158. Ahmed A, Lumb V, Das MK, Dev V, Wajihullah, et al. (2006 Prevalence of mutations associated with higher levels of sulfadoxine-pyrimethamine resistance in *Plasmodium falciparum* isolates from Car Nicobar Island and Assam, India. *Antimicrob Agents Chemother* 50: 3934-398.

159. Sowunmi A (2002) A randomized comparison of chloroquine, amodiaquine and their combination in the treatment of acute, uncomplicated, *Plasmodium falciparum* malaria in children. Annals of Tropical Medicine and Parasitology 96, 227–238.

160. Robert V, Awono-Ambene HP, Le Hesran JY, Trape JF (2000) Gametocytemia and infectivity to mosquitoes of patients with uncomplicated *Plasmodium falciparum* malaria attacks treated with chloroquine or sulfadoxine plus pyrimethamine. *Am J Trop Med Hyg* 62: 210-216.[Crossref]

161. Smalley ME, Sinden RE (1977) *Plasmodium falciparum* gametocytes: their longevity and infectivity. *Parasitology* 74: 1-8.[Crossref]

162. Sowunmi, A. and B.A. Fateye. 2002. Studies on *Plasmodium falciparum* gametocytaemia in children: peripheral immature gametocytaemia as a marker of response to chloroquine treatment in children from an endemic area. Am. Soc. Trop. Med. Hyg. 4: 10-14.

163. Price R, Nosten F, Simpson JA, Luxemburger C, Phaipun L, et al. (1999) Risk factors for gametocyte carriage in uncomplicated falciparum malaria. *Am J Trop Med Hyg* 60: 1019-1023.[Crossref]

164. Nacher M,Silachamroon U, Singhasivanon P, Wilairatana P, Phumratanaprapin W(2004) Comparison of artesunate and chloroquine activities against *Plasmodium vivax* gametocytes. *Antimicrob Agents Chemother* 48: 2751-2752.

165. Shulman CE, Dorman EK, Cutts F, Kawuondo K, Bulmer NJ(1999)Intermittent sulphadoxine-pyrimethamine to prevent severe anaemia secondary to malaria in pregnancy: A randomised placebo-controlled trial. *Lancet* 353: 632-636.

166. Schellenberg D, Menedez C, Aponte JA, Kahigwa E, Tanner M(2005) Intermittent preventive antimalarial treatment for Tanzanian infants: Follow-up to age 2 years of a randomised, placebo-controlled trial. *Lancet* 365: 1481-1483.

167. Price RN, Nosten F, Luxemburger C, ter Kuile FO, Paiphun L, et al. (1996) Effects of artemisinin derivatives on malaria transmissibility. *Lancet* 347: 1654-1658.[Crossref]

168. Doherty JF, Sadiq AD, Bayo L, Alloueche A, Milligan P(1999) A randomised safety and tolerability trial of artesunate plus sulfadoxine-pyrimethamine versus sulfadoxine-pyrimethamine alone for the treatment of uncomplicated malaria in Gambian children. *Trans R Soc Trop Med Hyg* 93: 543-546.

169. Hallett RL, Sutherland CJ, Alexander N, Ord R, Jawara M(2004) Combination therapy counteracts the enhanced transmission of drug-resistant malaria parasites to mosquitoes. *Antimicrob Agents Chemother* 48: 3940-3943.

170. Alker AP, Lim P, Sem R, Shah NK, Yi P, et al. (2007) Pfmdr1 and *in vivo* resistance to artesunate-mefloquine in falciparum malaria on the Cambodian-Thai border. *Am J Trop Med Hyg* 76: 641-647.[Crossref]

171. Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, et al. (2009) Artemisinin resistance in *Plasmodium falciparum* malaria. *N Engl J Med* 361: 455-467.[Crossref]

172. Hung le Q, Vries PJ, Giao PT, Nam NV, Binh TQ, et al. (2002) Control of malaria: a successful experience from Viet Nam. *Bull World Health Organ* 80: 660-666.[Crossref]

173. Thang NDEA, le Hung X, le Thuan K, Xa NX, Thanh NN, et al. (2009) Rapid decrease of malaria morbidity following the introduction of community-based monitoring in a rural area of central Vietnam. *Malar J* 3: 3-8.

174. Morrow M, Nguyen QA, Caruana S, Biggs BA, Doan NH, et al. (2009) Pathways to malaria persistence in remote central Vietnam: a mixed-method study of health care and the community. *BMC Public Health* 9: 85.[Crossref]

175. Anh NQ, Hung le X, Thuy HN, Tuy TQ, Caruana SR, et al. (2005) KAP surveys and malaria control in Vietnam: findings and cautions about community research. *Southeast Asian J Trop Med Public Health* 36: 572-577.[Crossref]

176. Thanh NV, Cowman AF, Hipgrave D, Kim TB, Phuc BQ, et al. (2001) Assessment of susceptibility of *Plasmodium falciparum* to chloroquine, quinine, mefloquine, sulfadoxine-pyrimethamine and artemisinin in southern Viet Nam. *Trans R Soc Trop Med Hyg* 95: 513-517.

177. Phuc BQCS, Cowman AF, Biggs BA, Thanh NV, Tien NT, et al. (2008) Prevalence of polymorphisms in DHFR, PFMDR1 and PFCRT genes of *Plasmodium falciparum* isolates in Quang Tri Province, Vietnam. Southeast Asian J. Trop. Med. Public Health. 39: 1-4.

178. Ngo T, Duraisingh M, Reed M, Hipgrave D, Biggs B, et al. (2003) Analysis of pfcrt, pfmdr1, dhfr, and dhps mutations and drug sensitivities in *Plasmodium falciparum* isolates from patients in Vietnam before and after treatment with artemisinin. *Am J Trop Med Hyg* 68: 350-356.[Crossref]

179. Sinden RE (1982) Gametocytogenesis of *Plasmodium falciparum* in vitro: ultrastructural observations on the lethal action of chloroquine. *Ann Trop Med Parasitol* 76: 15-23.[Crossref]

180. Sinden RE (1983) Sexual development of malarial parasites. *Adv Parasitol* 22: 153-216.[Crossref]

181. Sowunmi A, Fateye BA(2003)Asymptomatic recrudescent chloroquine-resistant *Plasmodium falciparum* infections in Nigerian children: clinical and parasitological

characteristics and implications for transmission of drug resistant infections. *Ann Trop Med Parasitol* 97: 469-479.

182. Sowunmi A, Salako LA (1992) Evaluation of the relative efficacy of various antimalarial drugs in Nigerian children under five years of age suffering from acute uncomplicated falciparum malaria. *Ann Trop Med Parasitol* 86: 1-8.

183. Sowunmi A, Oduola AMJ, Ogundahunsi OAT, Falade CO, Gbotosho GO, et al. (1997) Enhanced efficacy of chloroquine–chlorpheniramine combination in acute uncomplicated falciparum malaria in children. *Trans Royal Soc Trop Med Hyg* 91: 63-67.

184. Trape JF, Pison G, Preziosi MP, Enel C, Desgrées du Loû A, et al. (1998) Impact of chloroquine resistance on malaria mortality. *C R Acad Sci III* 321: 689-697.[Crossref]

185. Prajapati SK, Joshi H, Dev V, Dua VK (2011) Molecular epidemiology of *Plasmodium vivax* anti-folate resistance in India. *Malar J* 10: 102.[Crossref]

186. Butcher GA (1997) Antimalarial drugs and the mosquito transmission of Plasmodium. *Int J Parasitol* 27: 975-987.[Crossref]

187. Marfurt J, Smith TA, Hastings IM, Müller I, Sie A, et al. (2010) *Plasmodium falciparum* resistance to anti-malarial drugs in Papua New Guinea: evaluation of a community-based approach for the molecular monitoring of resistance. *Malar J* 9: 8.[Crossref]

Copyright: ©2016 Upadhyay RK. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.