Chapter 2

The Artemisinin Resistance in Southeast Asia: An Imminent Global Threat to Malaria Elimination

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Additional information is available at the end of the chapter

http://dx.doi.org/10.5772/intechopen.76519

Abstract

Malaria remains a leading cause of mortality and morbidity in many low- and middle-income countries. Artemisinin combination therapies (ACTs) have contributed to the substantial decline in the worldwide malaria burden, renewing the optimism that malaria elimination is achievable in some regions of the world. However, this prospect is threatened by the emergence of artemisinin resistance in *Plasmodium falciparum* leading to clinical failure of ACTs in Southeast Asia. Historically, drug resistance in *P. falciparum* has emerged in SEA and spread to Africa. Today, resistance to ACTs could reverse all the achievements of control and elimination efforts globally. With no new drug available, *P. falciparum* malaria must be eliminated from the Greater Mekong before it becomes untreatable.

Keywords: falciparum malaria, artemisinin, ACT, resistance, malaria elimination, Southeast Asia

1. Introduction

The emergence of artemisinin-resistant falciparum malaria along the Thai-Cambodian border follows a familiar pattern. History shows that chloroquine resistance had arisen from this region in the 1950s (**Table 1**) and leads to the failure of the Global *Malaria Eradication* Programme [1, 2] Resistance to artemisinin with concomitant emergence of partner drug resistance is now causing high artemisinin combination therapy (ACT) treatment failure rates in Cambodia, Vietnam, Thailand, Laos and Myanmar (**Table 1**). The prospect of untreatable malaria has once again loomed and threatened the effective malaria control and elimination efforts.

Antimalarial drug	Year of first deployment	Place of first deployment	Year of resistance emerged	Place of emergence of resistance
Quinine	1630 [34]	South America [34]	1910*	Brazil [28, 29]
Chloroquine	1945	Global Malaria Eradication Campaign [127]	1957	Colombia, Cambodia-Thailand border [41, 128–130]
Amodiaquine	1948	Americas [131, 132]	1961	Colombia [56, 57]
Atovaquone	1996	Thailand [73]	1996	Thailand [72, 73, 75]
Proguanil	1948	Various African countries [133]	1949	Aden Protectorate, Yemen [134]
Sulfa + antifols°	1967	Thailand [135]	1967	Thailand [135]
Mefloquine	1967	Vietnam [136]	1982	Thailand [7, 8, 43]
Piperaquine	1978	China [137]	1985	China [138]
Artemisinin	1979	China [139]	2008	Cambodia [6]
Mefloquine-artesunate	1994	Thailand [140]	2002[α]	Cambodia [141]
Artemether-lumefantrine	1994	China [142]	2006[α]	Cambodia [143, 144]
Dihydroartemisinin-piperaquine	2001	Cambodia [145]	2013[α]	Cambodia [86, 146, 147]

*There is no high-grade resistance to quinine.

[α]Therapeutic efficacy <90% (cut-off threshold of WHO to switch the ACT policy).

°Sulfa + antifols: Sulfadoxine + antifolates.

Table 1. Different antimalarial drugs and years/places of deployment and emergence of resistance [references in bracket].

2. Background

Resistance in *Plasmodium falciparum* has already developed to all antimalarial drug classes deployed for treatment. Paradoxically, the number of antimalarials available or in development has remained small. For most of the twentieth century, chloroquine was the main drug used to treat or prevent malaria. The discovery of chloroquine after World War II, and the widespread use of DDT for vector control, had triggered hope that malaria eradication was possible [3]. Unfortunately, chloroquine resistance did emerge and spread to the African continent within two decades annihilating the prospect of malaria eradication [4]. Although several countries did achieve malaria elimination (in Europe and the Americas), others saw a dramatic resurgence of the disease [3]. Over the following period, *P. falciparum* developed resistance to all antimalarial drugs, including sulfadoxine, pyrimethamine, mefloquine, atovaquone, artemisinin derivatives and piperaquine [5–8]. The most accurate and up-to-date data repository of the clinical trials on the efficacy of antimalarials, and the temporal and geographical spread of resistance is accessible at the Worldwide Antimalarial Resistance Network (WWARN: www.wwarn.org).

In 2007, the Bill and Melinda Gates Foundation announced that it was investing millions of dollars to revitalise the efforts of malaria elimination [9]. Ten years later, this seems to be an achievable goal since the global malaria burden has diminished (**Figure 1**), an encouraging

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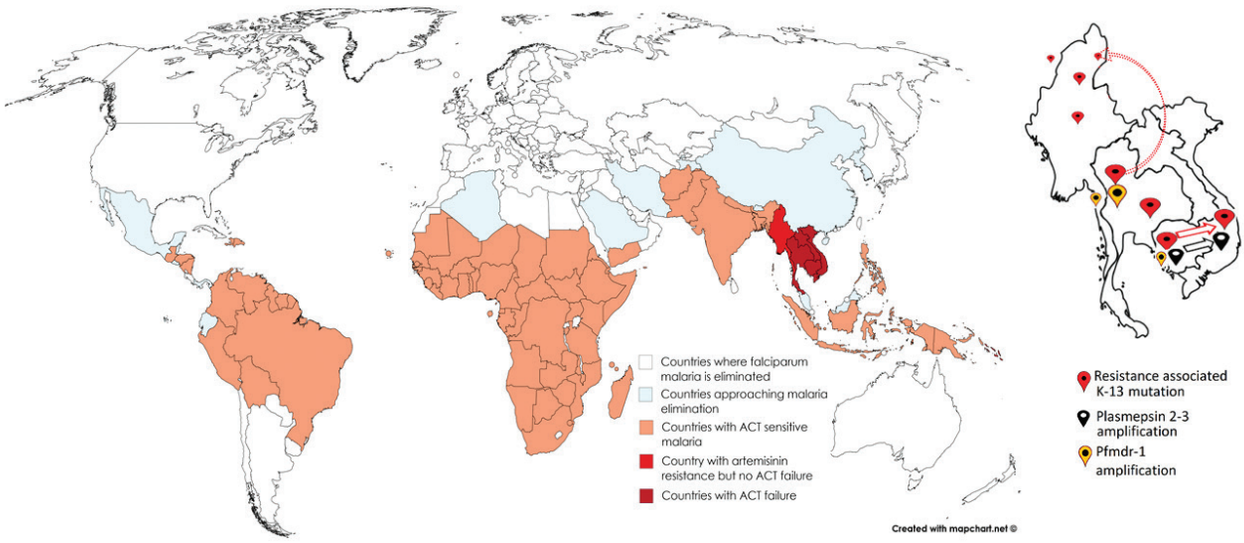

Figure 1. World atlas showing the countries with different stages of malaria endemicity [10] and status of drug resistance [121]. Right side: Prevalence (small pin: <10%, medium pin: 10–50%, large pin: >50% prevalence) of *Pfmdr-1* CNV [25, 122], Plasmepsin 2–3 CNV [26, 87], K-13 mutation [101, 123, 124] and possible spread [125, 126].

result attributed to the widespread deployment of long-lasting impregnated nets (LLINs), the ACTs and increased availability of malaria diagnostic tests [10]. However, the failure of the ACTs, the extension of vector resistance to the insecticides and the recent increase in the number of malaria cases are clear reminders that malaria is a formidable foe. Without new strategies, the same causes will lead to the same consequences [10] .

3. Mechanisms and emergence of antimalarial drug resistance

Causal stimuli of antimalarial resistance consist of spontaneous mutations in the parasite genome, antimalarial pharmacokinetics and the magnitude of parasite gene pool, which is proportionate to transmission intensity.

Primarily, as an innate survival strategy of microorganisms, mutation(s) occur *de novo*, independent of drug pressure. However, the parasite's genome replication rate, mutation rate per base-pair per parasite generation and the total number of parasites at any given time are the principal determinants in spontaneous mutation [11, 12]. These spontaneous mutations can be either minor scale modification, such as insertion, deletion or variation in a nucleotide (frameshift mutation or single-nucleotide polymorphism), or bulky transfiguration of large chromosomal regions (gene amplification/deletion/copy number variations). For some drugs, a single genetic event may be all that is required. A single point mutation in the parasite genome is sufficient to confer resistance (e.g. atovaquone), while for other drugs, multiple unlinked events (epistatic modulation) may be necessary (e.g. triple mutant in pyrimethamine [13, 14], Kelch-10, Kelch-13 and background mutations [15–17] in artemisinin resistance).

Spontaneous mutations, in the particular genes encoding the drug target, cause the reduction in drug accumulation or efflux (chloroquine, amodiaquine, quinine, mefloquine, halofantrine

resistance) or reduced affinity of the drug target (pyrimethamine, cycloguanil, sulphonamide, atovaquone resistance), which finally enables the parasite to withstand the antimalarial treatment. Afterwards, the drug pressure facilitates the resistant parasites to propagate by eliminating the susceptible parasites, which are usually more fit and would outcompete the resistant ones in the absence of the drug. Eventually resistance becomes established and can persist or be reintroduced. In the absence of drug pressure, the resistant parasites have no longer any survival advantage and can be overtaken by wild-type (sensitive) parasites [18, 19]. But as soon as the abandoned drug is reintroduced, the resistant isolates regain their survival advantage and expand rendering the drug inefficient within a short time [20].

Large-scale and/or long-term distribution of several tons of medicated salt took place in many countries and was an important factor implicated in the emergence of both chloroquine and sulfadoxine/pyrimethamine (SP) resistance and accelerating their spread [21–23]. In WHO supported programs, the doses of antimalarial received by each individual were highly variable, and constant exposure to sub-parasiticidal (or even parasiticidal) drug concentrations might have eliminated the highly and moderately sensitive parasites, providing a selective advantage for less sensitive counterparts. Thus, the speed of selection of mutant parasites depends principally on the pharmacokinetics of the drug (slowly eliminated drugs with a long tail of sub-parasiticidal concentrations generally select faster) and the magnitude of drug use within a population (the higher the drug pressure per parasite, the faster the selection).

With ACTs, the newly emerged drug-resistant parasite has to overcome the parasiticidal action of the partner drug as well as the host immunity. At this point, with compromised efficacy of partner drug, along with declining immunity of the population, resistance to ACT combination is inevitable [24]. This is the reason why artemisinin resistance has led to the clinical failure of mefloquine-artesunate and DHA-piperaquine combinations [25, 26].

The reason why antimalarial resistance always emerged in the same region of the world (SEA and specifically in Western Cambodia) is currently unknown. Some contributing factors have been proposed such as the low level of acquired immunity, the weak and seasonal transmission, the availability of antimalarial drugs, usage of monotherapies, sub-standard or counterfeit drugs, porous borders. The answer will probably be given by studies of the parasite population genetics, and recent work has shown the existence of "founding populations" favourable to the emergence of resistant parasites [17].

The emergence of drug resistance to various antimalarial compounds is mentioned by chronology in **Table 1** (antimalarial drugs and years/places of deployment and emergence of resistance).

3.1. Quinine resistance

Quinine, initially as cinchona bark, was first used as a fever medicine and officially introduced into the London Pharmacopoeia in 1677 [27]. The earliest resistance to quinine was reported in 1910 [28, 29]. Like chloroquine, quinine has been shown to accumulate in the parasite's digestive vacuole inhibiting the haem detoxification process. Quinine resistance also seems to be associated with reduced drug uptake by the parasite. There is a weak association between quinine resistance and *Pfmdr-1* amplification or *Pfmdr-1* SNP as well as Pf Na-H exchanger (*Pfnhe-1*) and *Pfcrt* [30, 31]; hence, it is probable that multiple genes are influencing susceptibility and probably in a strain-dependent manner. There were only a few *in vitro* data in Asia

[32], South America [33] and Africa [34] showing diverse range of sensitivities. However, the review paper of over 400 clinical trials showed that the failure rates for quinine (the only compound besides artemisinins, derived from nature) reported over the past 30 years remain steady and high grade clinical resistance to quinine is very rare [35].

3.2. Chloroquine resistance

Chloroquine, considered as one of the most successful medications ever deployed, saving several millions of lives, was developed in 1934 [2, 36] and replaced quinine for shorter regimen with better adherence. Single nucleotide polymorphisms in *Pfcrt* gene encoding for a transporter, chloroquine (CHQ) resistance transporter in the food vacuole causing the efflux of CHQ [37, 38], and acidification of the food vacuole [39] are significantly associated to CHQ resistance *in vitro* and are sensitive markers for therapeutic failure. Phylogenetic analysis revealed that a single lineage of CHQ-resistant *Pfcrt* alleles, that is, *CVIET*/*S* (K76T and mutations in three other amino acids, at positions 72, 74, 75 and 76) [40], which had emerged on the Thai-Cambodia border in 1957 [41], spread to India and Middle East countries between 1977 and 1987, reached West Africa in 1987 and propagated throughout the African continent leading to the death of millions of children [2, 38, 42, 43].

3.3. Antifolate resistance

After the emergence of chloroquine resistance, sulfadoxine-pyrimethamine (SP) combination was deployed by the Thai Malaria Control Program as the first-line regimen for falciparum malaria in 1973. Afterwards, SP was extensively used throughout the country and was also available as an over-the-counter fever remedy in local dispensaries. Attributed to a number of reasons, including unrestricted usage, distribution of pyrimethamine medicated salt [23], superfluous drug pressure (prophylactic as well as presumptive use for fever) and poor compliance especially in migrant mobile population, the resistance to SP combination had emerged around 1980 in the Thai-Cambodian border [5, 44]. Then, in the early 1980s, even with an increased dose (i.e. three tablets of SP, instead of two tablets flat dosing), a cure rate of only 30–40% was achieved [44].

Point mutations at codons 51, 59, 108 and 164 in the *dhfr* gene [45, 46] confer resistance to pyrimethamine; double or triple mutant resistant strains generated from sequential point mutations, based upon the common S108 N allele, are associated with 100-fold rise of *in vitro* sensitivity to pyrimethamine compared to wild-type [47]. Similarly, sulfadoxine resistance is associated with *DHPS* mutations at codons 436, 437, 581, 613 and 540 [48, 49]. Pyrimethamine resistant double mutant alleles (S108 N plus one more mutation at position 51 or 59) with low-level resistance of *dhfr* have multiple independent origins [50, 51]; by contrast, there were only a few or perhaps a single founding mutant lineage for the triple (N51I + C59R + S108 N) mutant *dhfr* allele, which originated from Southeast Asia (SEA) and spread to Africa [13, 14].

3.4. Amodiaquine resistance

Amodiaquine is structurally related to chloroquine but these amino-4-quinolines have different resistance patterns. Amodiaquine is effective against chloroquine-resistant isolates. However, parasites carrying the *CVIET* allele on the *Pfcrt* gene, as well as 86Y and 1246Y

polymorphisms on the *Pfmdr-1* gene, are resistant to amodiaquine [52–55]. The earliest report of resistance was documented since 1961 [56, 57], and widespread resistance to amodiaquine monotherapy was seen in 1980s [58].

3.5. Mefloquine resistance

Mefloquine was first produced in 1969 by the US Army Antimalarial Drug Development Program, primarily for the chemoprophylaxis in the military. The early therapeutic efficacy trial of mefloquine in Thailand showed 100% efficacy in 1976 [59] and in combination with SP where 97% efficacy was proven in a large-scale trial during 1983–1985 [60, 61]. Then, in 1991, mefloquine monotherapy was used as the first line regimen for *P. falciparum* malaria in Thailand [62]. Even with the stringent regulatory measures in Thailand, the therapeutic efficacy of mefloquine fell hastily especially in the border areas [7, 63]: because of the difficulties in restricting all access to the drug which was available across neighbouring porous borders. Then, in 1992, the cure rate of mefloquine monotherapy had fallen to 49% with 16% of high-grade failures in children [7, 63].

Resistance to mefloquine was proven to be mediated by *Pfmdr-1* gene amplification. *Pfmdr-1* is the gene encoding a transporter pump, P-glycoprotein homologue 1 (*Pgh1*), localised at the surface of the digestive vacuole of parasite (**Figure 2**). It confers drug resistance through both gene copy number variation (CNV) and point mutation (at nucleotide level). Altering the gene copy number provides a modest way to change gene expression without affecting the

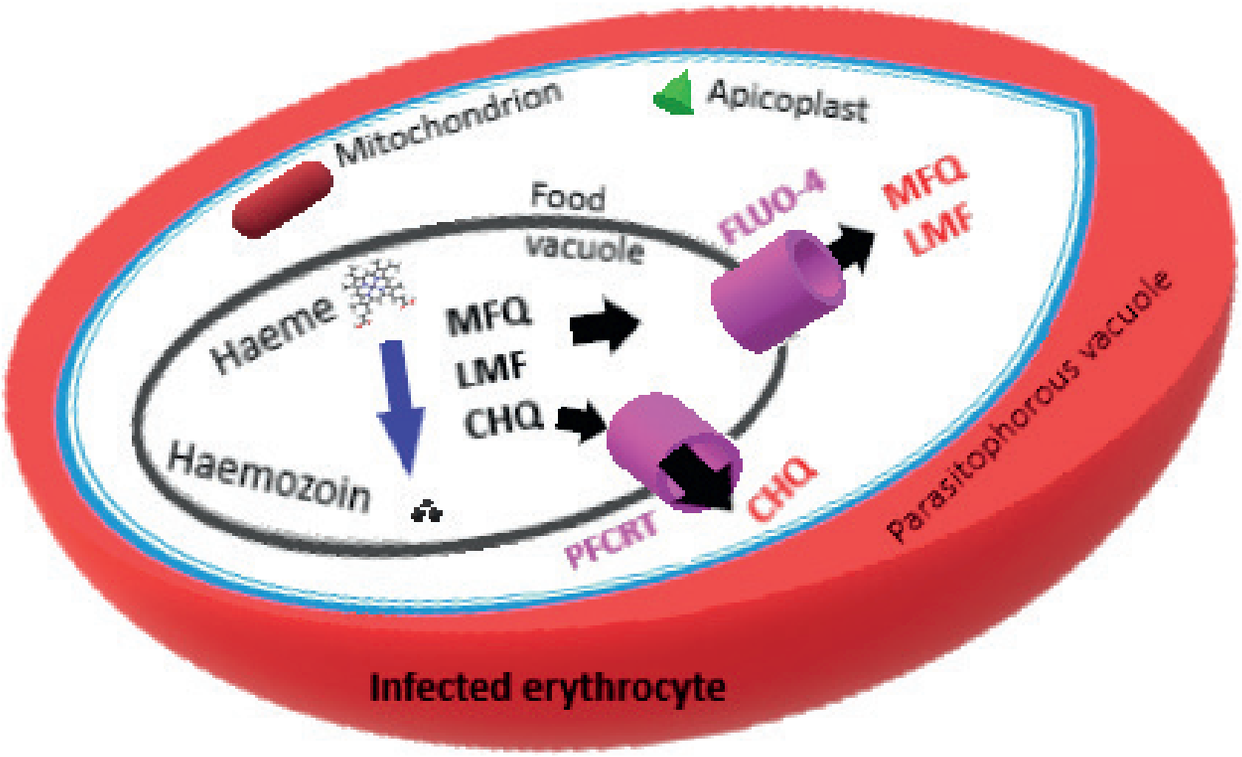

Figure 2. *Pfmdr-1* gene and mechanism of Pgh-1 pump. MFQ – mefloquine, LMF – lumefantrine, CHQ – chloroquine and RBC – red blood cell.

nucleotide sequence [64]. Increased *Pfmdr-1* copy number is a significant independent risk factor for recrudescence in patients treated with mefloquine containing therapy [65–67] as well as *in vitro* mefloquine resistance [68]. *Pfmdr-1* gene amplification can be selected *in vitro* by exposing the parasites to stepwise increasing concentrations of mefloquine [69]. Reciprocally, reducing the copy number from isolates with multiple copies resulted in increased *in vitro* sensitivity of isolates to mefloquine, lumefantrine, halofantrine, quinine and artemisinin due to reduced transcription and encoding of *Pgh-1* pump [70]. This is also true for the clinical efficacy since the rise and fall of amplified *Pfmdr-1* prevalence is temporally associated with the deployment of mefloquine in Cambodia [65, 71]. Along the Thailand-Myanmar border, patients infected with parasites having both *Pfmdr-1* multiple copy number and K-13 mutation were 14 times more likely to get recrudescence compared to the patients infected with wild-type infections [25].

3.6. Atovaquone resistance

Atovaquone was trialled as a monotherapy as well as in combination with proguanil between 1990 and 1996 in Thailand, and the therapeutic efficacy of atovaquone-proguanil was proven to be superior to mefloquine monotherapy, chloroquine, amodiaquine monotherapy and SP [72, 73]. A single point mutation (codon 268 in the *cyt-b* gene) in the ubiquinol oxidation region of cytochrome b confers atovaquone resistance *in vivo* [74, 75]. Generally, resistance conferred by a single point mutation can be rapidly acquired both *in vivo* and *in vitro,* and once the mutation is acquired, resistance becomes complete. Thus, not very long after deployment, atovaquone-resistant parasites could be selected *in vitro* after 5 weeks of continuous culture [76, 77]. In addition, atovaquone-resistant parasites were also resistant to the synergistic effects of proguanil [78], suggesting that once atovaquone resistance arises, the atovaquone-proguanil combination (Malarone) will be ineffective since cycloguanil (proguanil) resistance is already established in most malaria endemic areas.

3.7. Pyronaridine resistance

Pyronaridine is a quinoline derivative compound with similar molecular structure as chloroquine and amodiaquine. There was a strong correlation between *in vitro* sensitivity of pyronaridine and that of amodiaquine and halofantrine [79]. *Ex vivo* data indicated that there is an association between reduced susceptibility to pyronaridine and K76 T polymorphism in *Pfcrt* gene. However, there are scanty data on clinical trials and no confirmed report of molecular marker of pyronaridine resistance has been documented. Pyronaridine-artesunate combination had been granted a positive scientific opinion by the European Medicines Agency, removing all restrictions on repeat dosing with a condition to use only in areas of high resistance and low transmission, and has been included in WHO's list of prequalified medicines [80]. However, day-42 cure rate of <90% in Western Cambodia has challenged the expediency of the pyronaridine-artesunate combination in ACT resistance setting [81].

3.8. Piperaquine resistance

Piperaquine (PPQ) has no cross resistance with chloroquine, and susceptibility is not associated with mutations on the *Pfcrt* gene [82, 83]. PPQ resistance is inversely correlated with

mefloquine resistance *in vitro* and hence with *Pfmdr-1* copy number amplification [84–86]. Later findings have shown that the amplification of *Plasmepsin-2* gene (probably *Plasmepsin-3* as well) on chromosome 14 is significantly associated with piperaquine resistance *in vitro* as well as *in vivo* [26, 87]. Worryingly, a recent study in Cambodia has demonstrated the presence of parasite isolates with amplification of both *Pfmdr-1* and *plasmepsin-2* genes [20]. This finding indicates that the parasite has successfully adapted to acquire concomitant mutations related to resistance to these two different antimalarial partner drugs [20].

3.9. Artemisinin resistance

Artemisinins are thought to be inhibitor of *P. falciparum* phosphatidylinositol-3-kinase (*PfPI3K*), which phosphorylates phosphatidylinositol to produce phosphatidylinositol 3-phosphate involved in cell survival pathways. Hence, inhibition of *PfPI3K* activity causes a reduction in PI3P level, which subsequently leads to parasite death. After the introduction of artemisinins in the 1990s, the unanimous opinion by the experts was that resistance was unlikely to emerge because of inherent pharmacokinetic-dynamic property of the molecule. However, artemisinins were not everlasting drugs and the artemisinin resistance did emerge in 2008 [6].

There are two main proposed pathways for artemisinin resistance with the involvement of Kelch (*K-13*) mutations, that is, a cell survival signalling pathway with *PfPI3K* and an unfolded protein response pathway (*UPR*) [88].

In Kelch (propeller) mutant alleles, the mutations may alter the topology of the Kelch protein probably by modification of surface charges that disrupt interactions with other enzymes such as *PfPI3K* [89]. This leads to a reduced amount of ubiquitination, as well as degradation of *PfPI3K* associated with increased levels of both the enzyme *PfPI3K* and the substrate *PI3P* [90, 91]. The *PI3P* facilitating the host remodelling is present in the apicoplast and food vacuole and contributes to the cell survival pathways either through redox, transcriptional or DNA repair [90–94]. All of which have been implicated in artemisinin resistance [90, 95–98].

Possible mechanisms proposed by transcriptomic study [99] is through upregulation of genes involved in the UPR pathway (especially two putative chaperonin complexes, *Plasmodium* reactive oxidative stress complex/PROSC and TCP-1 ring complex/TRiC) which enhances the capacity of parasites to quickly repair or degrade proteins or other cellular components. (The UPR pathway is usually damaged by brief artemisinin exposures in patients, but these genes are upregulated in artemisinin resistant parasites) and/or downregulation of genes involved in DNA replication, which is associated with developmental arrest and dormancy [100].

The role of Kelch non-propeller mutation (before the amino acid position 441) is still unclear. Some SNPs like E252Q emerged earlier along the Thai-Myanmar border and associated with reduced efficacy of ACT [25] but are being taken over by the propeller SNPs particularly C580Y [101]. All these findings indicate that artemisinin resistance is likely to be multi-locus and that other genetic changes, such as P623T polymorphism in *Kelch-10* gene [15] and background mutations (arps10-apicoplast ribosomal protein S10, *Pfmdr-2*, ferredoxin, *Pfcrt* [17], etc.), are providing compensatory fitness for K-13 mutant parasites or perhaps conferring partner drug resistance.

http://dx.doi.org/10.5772/intechopen.76519

4. Resistance facilitates the transmission potential

For the newly selected resistant parasites to be propagated, the recrudescent infection is essential [102]. The threshold for successful transmission of malaria is around six viable gametocytes in one blood meal [103]. Post-treatment gametocytaemia is a composite of ongoing gametocytogenesis despite treatment (especially with ineffective drug) and the release of sequestered gametocytes, which is enhanced by drug-induced stress [104]. If the malaria infection is treated with partially effective drugs, post-treatment gametocytaemia is more likely. This was clearly shown for drugs such as CHQ and SP [105] as evidenced in patients with slower parasite clearance after artesunate treatment [106]. Moreover, mutant isolates were also related to pre- and post-treatment gametocytaemia [107–110] and hence possess transmission advantage (**Figure 3**).

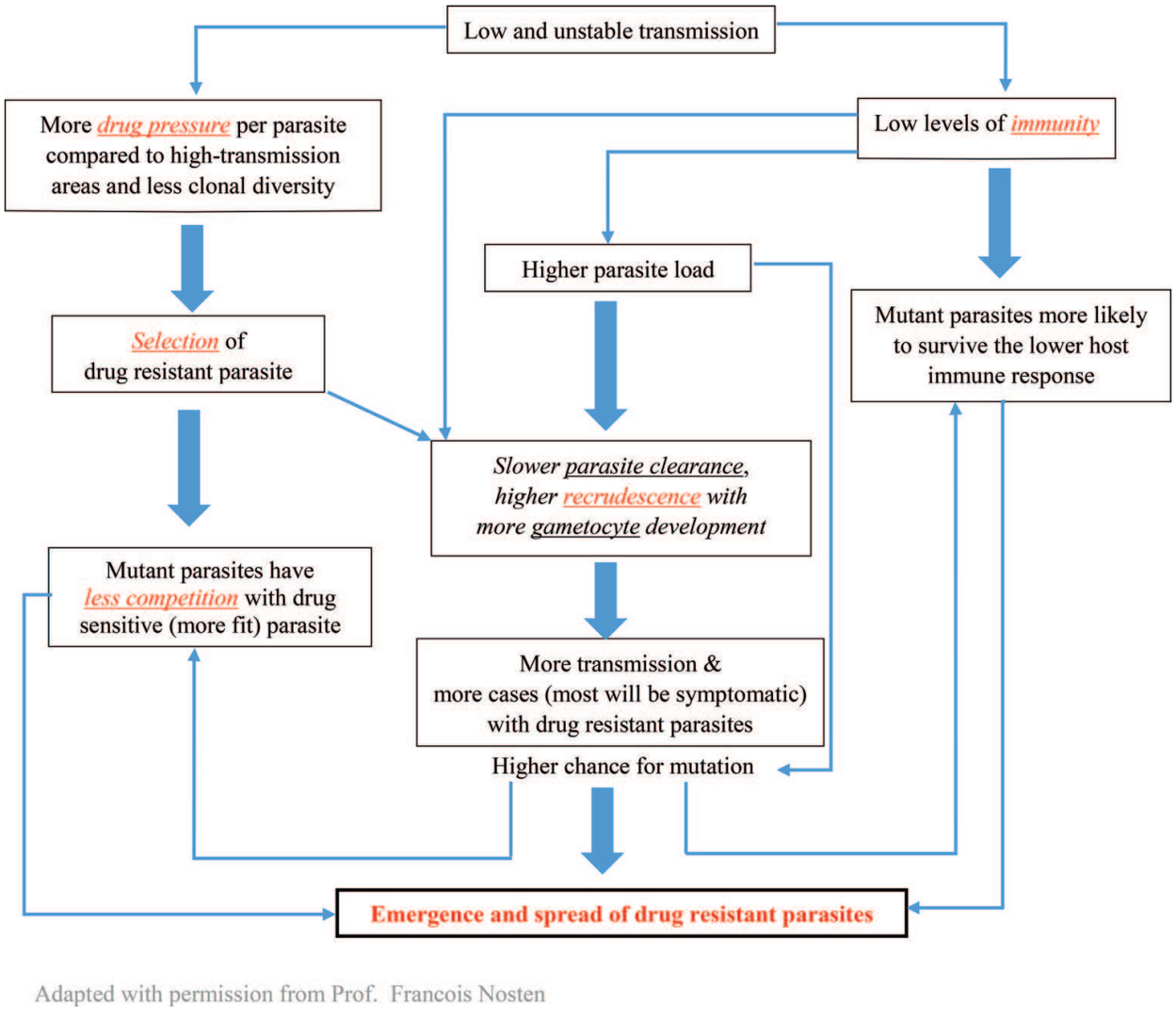

Figure 3. Postulated flow chart of emergence/spread of drug resistance (copyright permission from Prof Francois Nosten).

5. Prospects of elimination

With the declining transmission of malaria, the geographic clustering of both clinical and asymptomatic infections has become more apparent. Asymptomatic carriers represent a "reservoir" of parasites that are difficult to detect because the density of parasites is often below the sensitivity threshold of conventional diagnostic tools (Rapid Diagnostic Tests and microscopy). The size of these reservoirs of sub-microscopic infections (also called "hot-spots") can vary from a few households to large geographical areas. Clustering of these hotspots becomes more pronounced as transmission declines [111]. While considering malaria elimination, radical depletion of parasite reservoir (asymptomatic carriers with sub-microscopic parasitaemia) and gametocytes is a necessity. This can be achieved by two functional components: (1) early diagnosis with treatment (EDT) of the symptomatic patients (preferably within 48 hour of symptoms before the development of gametocytaemia) and (2) early detection and treatment targeting the reservoirs of sub-microscopic infections through Mass Drug Administration (MDA) [112, 113].

The intervention for the first element is to set up or reinforce and sustain malaria control program hence reducing the number of clinical episodes as much as possible through increased access to EDT where the use of efficacious antimalarial regimen is critical [114]. As the drug resistance worsen, the rising number of clinical cases due to increasing gametocyte carriage in the community will be inevitable. MDA or mass screening and treatment (MSAT) is only accelerating the malaria elimination alongside EDT, by eliminating the sub-microscopic reservoir [115]. The effectiveness of MDA or MSAT significantly relies on the therapeutic efficacy of the drug in use, the coverage and the total number of rounds of MDA. In turn, this means that a careful and well-conducted community engagement is primordial for enhanced coverage [115, 116].

6. Choice of drug for malaria elimination: is the pipeline empty?

The current malaria elimination program along the Thai-Myanmar border is using artemether-lumefantrine (AL) for treating the clinical cases at the village malaria posts or by malaria workers [114], whereas dihydroartemisinin-piperaquine (DP) is deployed in MDA activities [117]. In this area, the third ACT, mefloquine-artesunate combination, is already failing [25], and the prospect of elimination program is highly dependent on the therapeutic efficacy of AL and DP. Recent emergence of piperaquine resistance following the artemisinin resistance has depleted the available ACTs to be deployed in malaria elimination programs. High failure rates of AL in Laos PDR and DP in Vietnam and Cambodia have cast doubts on the optimism of malaria elimination [10, 26, 87, 118].

There are very few new compounds in the development pipeline. The front runners are OZ439, a synthetic endoperoxide, structurally related to artemisinin, and KAF156 belonging to a new class of antimalarial (imidazolopiperazines) and the spiroindolone cipargamin (formerly KAE609). However, these short-acting drugs will have to be deployed in combination therapies and their full development will take many years.

http://dx.doi.org/10.5772/intechopen.76519

As a stopgap measure, two triple ACTs (mefloquine plus DP and amodiaquine plus AL) are under multicentre trial, using the inverse correlation between susceptibility to amodiaquine and lumefantrine as well as between piperaquine and mefloquine. The trial has completed the patient recruitment and the results are promising with high cure rates. However, recent increasing prevalence of parasite isolates with potential resistance to both mefloquine and piperaquine has questioned the longevity of the triple ACT [20].

7. Drug resistance in *P. vivax*

For the *P. vivax*, chloroquine remains the first line of treatment in majority of the endemic countries. However, after the first report from Papua New Guinea in 1989, chloroquine resistance has reached northern Papua and Indonesia. Later on, data with recurrences (by day-28 of chloroquine treatment) greater than 10% have also been reported from Myanmar, Thailand, Cambodia, India, Vietnam, Turkey, South America, Ethiopia and Madagascar [119]. Resistance in *P. vivax* is more difficult to document than for *P. falciparum* because of the relapses from liver stages. The most robust proof of resistance is given when a circulating parasite is detected in the peripheral blood in the presence of therapeutic chloroquine concentrations (i.e. >100 ng/ml). The absence of long-term parasite culture for *P. vivax* further complicates the efficacy testing in the laboratory, but short-term assays have been developed in recent years.

8. Regional artemisinin resistance initiative (RAI)

The six countries of the Greater Mekong Subregion (GMS), Thailand, Myanmar, Cambodia, Laos, Vietnam and China (Yunnan Province), are part of a larger community, the Association of Southeast Asian Nations (ASEAN). Despite political pledges to fight artemisinin resistance and eliminate malaria, coordination remains hampered by deep political, economic and geographical gaps. The WHO strategic plans to counter artemisinin resistance failed to prevent its spread to the entire sub-region. In 2013, the Global Fund launched the Regional Artemisinin-resistance Initiative to provide financial support to the five countries affected by this new treat. This initiative came in addition to the contributions of the Global Fund to the Malaria National Program and contributed to the decrease in malaria-related mortality and morbidity in the region. However, these efforts have been compromised by the fragmentation in the public health policies, the disparities in the infrastructures and human resources as well as corruption. In terms of treatment policies, all GMS countries had already adopted ACTs long before the emergence of resistance, but poor monitoring in some countries meant that monotherapies and sub-standard or counterfeit drugs continued to circulate until recently. The relative absence of entomological data in some parts of SEA explains that there is no coherent strategy for containment of local disease vectors. Large budgets continue to be spent on long-lasting impregnated nets (LLINs) despite the absence of evidence of their effectiveness.

9. Conclusions

Artemisinin resistance in *P. falciparum* has emerged 10 years ago in SEA and spread in the entire GMS. Parasite populations resistant to all ACTs are now circulating in Cambodia, triggering a resurgence of the disease. Current gains in malaria control/elimination program are heavily relying upon the efficacy of ACTs. The emergence of artemisinin and partner drug resistance is a serious threat to the global prospect of malaria elimination. The recent decline in the number of clinical cases in the region is encouraging but by no means a victory. Current resurgence of malaria in Cambodia and the existence of large reservoirs of sub-microscopic infections must be seen as warnings that malaria could make a devastating comeback. Efforts must continue and accelerate to eliminate the parasite and this will only be possible with stronger political will and sustained financial support. The three main programmatic components are EDT, elimination of the reservoirs and adapted vector control measures. The few antimalarials in the development pipeline are promising, though these compounds will not be ready on time to replace the ACTs [120]. The spread of the ACT-resistant malaria has so far outpaced the malaria containment measures and time is running out. There are not many options but to accelerate the current malaria elimination efforts.

Acknowledgements

We acknowledge the insightful suggestions of two editors from InTech Open Access. The Shoklo Malaria Research Unit and Myanmar Oxford Clinical Research Unit are part of the Mahidol Oxford Tropical Medicine Research Unit-Tropical health network funded by the Wellcome Trust.

List of acronyms

ACT	artemisinin combination therapy
AL	artemether-lumefantrine
ASEAN	Association of Southeast Asian Nations
CNV	(gene) copy number variation
CHQ	chloroquine
DDT	dichlorodiphenyltrichloroethane
DP	dihydroartemisinin-piperaquine
EDT	early diagnosis and treatment
GMS	Greater Mekong Subregion
K-13	Kelch 13 gene of *P. falciparum*

LLINs	long-lasting impregnated nets
LMF	lumefantrine
MDA	mass drug administration
MFQ	mefloquine
MSAT	mass screening and treatment
Pfcrt	*P. falciparum* chloroquine resistance transporter
Pfmdr-1	*P. falciparum* multi-drug resistant gene-1
PfPI3K	*P. falciparum* phosphatidylinositol-3-kinase
Pgh1	P-glycoprotein homologue 1
RBC	red blood cell
SEA	Southeast Asia
SNP	single nucleotide polymorphism
SP	sulfadoxine-pyrimethamine
UPR	unfolded protein response pathway (UPR)
WWARN	Worldwide Antimalarial Resistance Network
WHO	World Health Organisation

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