**Artemisinin and multidrug-resistant *Plasmodium falciparum* – a threat for malaria control and elimination**

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**Purpose of review**
Artemisinin-based combination therapies (ACTs) are globally the first-line treatment for uncomplicated *falciparum* malaria and new compounds will not be available within the next few years. Artemisinin-resistant *Plasmodium falciparum* emerged over a decade ago in the Greater Mekong Subregion (GMS) and, compounded by ACT partner drug resistance, has caused significant ACT treatment failure. This review provides an update on the epidemiology, and mechanisms of artemisinin resistance and approaches to counter multidrug-resistant falciparum malaria.

**Recent findings**
An aggressive malaria elimination programme in the GMS has helped prevent the spread of drug resistance to neighbouring countries. However, parasites carrying artemisinin resistance-associated mutations in the *P. falciparum* Kelch13 gene (*pfk13*) have now emerged independently in multiple locations elsewhere in Asia, Africa and South America. Notably, artemisinin-resistant infections with parasites carrying the *pfk13 R561H* mutation have emerged and spread in Rwanda.

**Summary**
Enhancing the geographic coverage of surveillance for resistance will be key to ensure prompt detection of emerging resistance in order to implement effective countermeasures without delay. Treatment strategies designed to prevent the emergence and spread of multidrug resistance must be considered, including deployment of triple drug combination therapies and multiple first-line therapies.

**Keywords**
artemisinin, epidemiology, *Plasmodium falciparum*, resistance, treatment

**INTRODUCTION**
Deployment of artemisinin-based combination therapies (ACTs) along with vector control interventions have contributed to an important reduction in the global burden of *Plasmodium falciparum* malaria [1]. ACTs consist of a highly potent, fast-acting artemisinin derivative (artemether, artesunate or dihydroartemisinin) with a short plasma half-life, and a slower acting partner drug (lumefantrine, amodiaquine, mefloquine, piperaquine, sulphadoxine-pyrimethamine or pyronaridine) that remains in the blood for longer periods. Antimalarial treatment with artemisinins results in a 10 000-fold reduction in parasite load per 48-h erythrocytic life cycle. After a 3-day ACT course, any surviving parasites are then cleared by the partner drug [2]. Artesunate plus mefloquine (ASMQ) was the first ACT to be introduced over 25 years ago in north-western Thailand along the border with Myanmar in a context where multidrug-resistant *falciparum* malaria had become difficult to treat [3].

Increasing resistance to chloroquine and sulphadoxine-pyrimethamine in sub-Saharan Africa, bearing more than 90% of the world’s malaria burden, eventually led to ACTs becoming the treatment of choice for uncomplicated *P. falciparum* malaria in all endemic countries, as recommended by the WHO...
since 2006 [4,5]. In 2010, parenteral artesunate was recommended as the first-line treatment for both paediatric and adult severe malaria after large clinical trials showed the significantly reduced mortality in patients receiving artesunate compared with those receiving quinine [6–8].

Combining two drugs with different mechanisms of parasiticidal action provide mutual protection against the emergence of drug resistance in parasite populations [2]. However, artemisinin derivatives were used as monotherapies in South-East Asia (SEA) for decades until their use was discouraged in 2006 and is likely to have continued in the years thereafter [4,9]. Use of artemisinin monotherapies and of partner drugs at subtherapeutic doses and/or in substandard formulations has likely contributed to the emergence of artemisinin resistance (ART-R) in SEA, first described in western Cambodia in 2008 [10–12]. ART-R is characterized by reduced killing of ring-stage *P. falciparum*, resulting in delayed parasite clearance down to 100-fold per 48-h parasite life cycle [13]. In the Greater Mekong Subregion (GMS), ART-R has been compounded by ACT partner drug resistance, causing high treatment failure of ACTs. Dihydroartemisinin-piperaquine failure rates reached high levels in Cambodia and southern Vietnam, whereas ASMQ failed in the Myanmar-Thai border region [14,15]. In the GMS, all of the six available ACTs have shown efficacy below 90% at some point of time.

In this review, we will describe recent advances in the understanding of ART-R, its evolving epidemiology and strategies to treat multidrug-resistant falciparum malaria in order to combat its further emergence and spread.

**CLINICAL PHENOTYPE OF ARTEMISININ RESISTANCE**

An early study of ART-R demonstrated that peripheral blood parasitaemia was cleared more slowly in patients with recrudescent infections after ACT treatment [12]. The parasite clearance time or the proportion of patients that are still parasitaemic by light microscopy 72 h after start of treatment is used for surveillance of ART-R, but is confounded by multiple factors including the starting parasitaemia [16]. A slower parasite clearance rate provides a more accurate measure of ART-R, as it is largely independent from the ACT partner drug and initial parasitaemia [17]. The parasite clearance rate is estimated from the slope of the linear part of the log-linear parasite clearance curve, assessed by frequent light microscopy assessments of the peripheral blood parasite densities, and is described by the parasite clearance half-life (PC1/2) [18]. A PC1/2, more than 5 h has been used to define ART-R in Southeast Asia, though a threshold of more than 5.5 h has also been proposed [19,20]. In regions of high malaria transmission such as in many parts of sub-Saharan Africa, human host immunity will accelerate parasite clearance [21], and the threshold is likely lower. An alternative threshold for these settings, however, has not yet been validated.

**MECHANISMS OF ARTEMISININ RESISTANCE**

Although several specific parasite targets for artemisinins have been described, it is likely that the parasiticidal effect of artemisinins is mediated through alkylation of multiple cellular proteins and lipids. Given the broad scope of attack on the parasite and the diversity of ART-R parasite populations, it has proven to be difficult to define with precision a unifying mechanism of resistance to artemisinins. However, a few common interwoven themes have emerged from the extensive laboratory and omics-based research conducted to decipher the mechanism of ART-R (reviewed in [22**,23]). First, the progression through the asexual intracellular development cycle of ART-R parasites appears to be altered. This property of ART-R parasites was first identified as quiescence in cultured parasites exposed to increasing doses of artemisinin and eventually aided the discovery of mutations in the propeller domains of *P. falciparum* Kelch13 gene (*pfk13*) as genetic markers for ART-R [24,25]. Close to 100 *pfk13* mutations have since been described, of
which 10 are currently listed as validated markers for artemisinin resistance, whereas another 11 are associated or candidate markers [26]. A corollary from this work was the observation that early ring stages of ART-R parasites had an increased ability to survive the transient attack from the short-lived artemisinins [27]. This is corroborated by the inverse relationship between PC\textsubscript{1/2} and increasing parasite age at treatment observed in infections with parasites carrying \textit{pfk13} mutations known to confer ART-R [28]. Second, although transfection of \textit{P. falciparum} with a number of \textit{pfk13} mutations has proven a causal relationship between these mutations and ART-R \textit{in vitro} [29], establishing these mutations within the parasite populations in the field likely requires a genetic backbone of supportive mutations, possibly to compensate for a loss in fitness of \textit{pfk13} mutant parasites [30]. This genetic backbone was observed in ART-R parasites in the GMS, but the same background mutations were not detected in the more recently described ART-R parasite population in Rwanda [31]. Further, there can be significant divergence in the genomic, transcriptomic and proteomic profiles even within ART-R parasite populations with validated \textit{pfk13} mutations [32,33]. However, these profiles, reflecting the upregulation and downregulation of different cellular pathways, do converge into a distinct number of cellular responses to artemisinin exposure. These responses include an enhanced unfolded protein or cellular stress response and degradation of damaged protein in proteasomes as well as diminished or arrested transcription, translation and metabolism in ring-stage parasites, delaying progression through the asexual life cycle. The reduced trafficking and metabolism of haemoglobin in particular may in itself reduce the activation, and hence effectiveness, of artemisinins [22]. Finally, although some studies suggest putative drugs for coadministration with ACTs to reverse the effects of ART-R, none have so far been shown to be effective in treatment of patients with multidrug-resistant malaria [33,34].

**Epidemiology of Artemisinin Resistance**

In areas with \textit{P. falciparum} resistant to both the artemisinin and ACT partner drug, recrudescence rates will be very high, as observed in Cambodia and Vietnam after dihydroartemisinin-piperaquine treatment [14,35,36]. This caused a very rapid spread throughout Cambodia towards Vietnam of a single parasite lineage resistant to both artemisinins and piperaquine [37,38]. In the absence of partner drug resistance, the majority of ART-R infections can still be cleared adequately. However, in these infections, a much higher remaining parasite biomass after 3 days of ACT therapy will be exposed to only the ACT partner drug, because of the very short plasma half-life of the artemisinins. This facilitates the emergence of resistance to the partner drug. Such sequential acquisition of multidrug resistance has been observed in SEA with ASMQ [15], and dihydroartemisinin-piperaquine [14], although piperaquine resistance might have been present in Cambodia at the moment of dihydroartemisinin-piperaquine deployment. Further, whereas artemisinins are gametocytocidal for stage I to early-stage V gametocytes in susceptible parasite strains, ART-R causes gametocytes to become less susceptible and possibly also promotes gametocytogenesis [19,39,40]. In general, gametocyte carriage is increased in recrudescent \textit{P. falciparum} infections, amplifying the transmission of resistant parasites when ACT efficacy starts to dwindle (Fig. 1) [40]. These factors confer a selective advantage for ART-R and in particular multidrug-resistant parasites, expanding their prevalence in the parasite population, especially in regions with low malaria transmission [37].

Mutations in the propeller region of the \textit{pfk13} gene, which confer ART-R have emerged in multiple locations within and outside of the GMS (Fig. 2) [41,42,43,44]. In the eastern GMS, several \textit{pfk13} mutations associated with increased PC\textsubscript{1/2} emerged on a common genetic background [19,30] and converged to a single lineage of parasites carrying the \textit{pfk13} C580Y mutation. This allele has become...
predominant in Cambodia, Laos and Vietnam, assisted by the acquisition of mutations conferring piperazine resistance (*P. falciparum* plasmepsin 2/3 gene amplification and novel *pfcr* mutations). The predominance of the C580Y mutation occurred despite the relatively higher fitness cost of the C580Y mutation in vitro as compared to some other *pfk13* mutants [45], and was likely facilitated by continued drug pressure from dihydroartemisinin-piperazine as first-line antimalarial treatment providing a selective advantage for these parasites [37]. In Myanmar, multiple *pfk13* mutants, including an independently emergent C580Y allele, have been detected but parasites with the F446I allele are

![FIGURE 2. Spread of *pfk13* haplotypes across the Greater Mekong Subregion. The long *pfk13* C580Y haplotype that emerged in 2008 spread from its origin in western Cambodia to Thailand, Laos and Viet Nam. The blue arrows depict a single *pfk13* F446I haplotype that probably originated in northern Myanmar. Adapted from [41].]
now the most widespread [41**]. The increased prevalence of this validated marker for ART-R, which confers an intermediate phenotype of moderately prolonged parasite clearance but potentially increased transmissibility, has currently not led to higher treatment failure rates with artemether-lumefantrine, which is the first-line treatment in Myanmar. The F446L allele appears to have emerged in north-western Myanmar before spreading throughout Myanmar and also into India [46]. A study from West Bengal in India reported pfk13 mutations identified from locally acquired or imported cases, but these findings have not been confirmed in later studies [47,48*]. However, vigilant surveillance of antimalarial drug resistance markers in India remains important as it has in the past been the corridor of spread of resistance to chloroquine and sulphadoxine-pyrimethamine from the GMS to Africa.

Although there is no further evidence thus far that pfk13-mediated ART-R has spread beyond the GMS, de-novo emergence of ART-R has been clearly documented more recently. Most worryingly, P. falciparum carrying a validated marker of ART-R, pfk13 R561H, has emerged and expanded in Rwanda [31*]. Analysis of the microsatellites in the flanking regions of the pfk13 gene showed a common origin for this parasite population, different from the RS61H haplotype identified in the GMS. This Rwandan ART-R parasite lineage shows the phenotypic hallmarks of ART-R, that is delayed parasite clearance in vivo along with increased survival after in vitro exposure to dihydroartemisinin [49**], but has fortunately not as yet affected the efficacy of artemether-lumefantrine, the first-line ACT in Rwanda. ART-R associated pfk13 mutations, which have emerged independently from the GMS have also been observed in Guyana [50], New Guinea [51] and Uganda [52,53]. The pfk13 C580Y mutation in Guyana were found in surveys between 2010 and 2017, but its prevalence has decreased. In Uganda, an increasing prevalence of the A675V mutation was observed over time. A low prevalence, typically less than 3%, of pfk13 mutant P. falciparum has been described in multiple studies from sub-Saharan Africa, without evidence of selection, likely representing the background mutation rate in the pfk13 gene and also mutations not associated with ART-R such as the pfk13 A578S mutation [30]. In addition, there have been recent reports of decreasing artemether-lumefantrine efficacy detected in Angola and Burkina Faso that however appear to be unrelated to ART-R [54,55]. All this emphasizes the need for continued and sufficiently granular surveillance for ART-R and ACT partner drug resistance in the African setting [42**,46].

### Treatment of Artemisinin-Resistant Malaria

ACTs continue to be the only widely deployable treatment option to treat uncomplicated falciparum malaria, even in the GMS context wherein ART-R is highly prevalent. Five ACTs are currently recommended by the WHO: artemether-lumefantrine, ASMQ, dihydroartemisinin-piperaquine, artesunate-amodiaquine and artesunate-sulphadoxine-pyrimethamine [8]. In consideration of recent positive scientific opinion regarding its safety and efficacy, the WHO now also recommends the ACT artesunate-pyronaridine [56]. The current strategy to address confirmed ACT failure of more than 10% as assessed in therapeutic efficacy studies is to cycle through these ACTs based on the prevalent sensitivity of parasites to partner drugs [26]. In Cambodia, first-line antimalarial treatment shifted from ASMQ in 2000 to dihydroartemisinin-piperaquine in 2008 back to ASMQ in 2016 [14,36,57]. This strategy has however been logistically challenging. Delays in implementation led to patients being treated with an inferior ACT and spread of multidrug-resistant parasites, threatening region-wide malaria elimination efforts. New strategies to treat ART-R malaria are needed to circumvent these challenges.

A number of promising new antimalarials are in clinical development [58*]. Some of these including, ciapergamine (KAE609), a spiroindolone that acts even more rapidly than artemisinin and ganaplacide (KAF156), an imidazolopiperazine acting on different stages of the parasite life cycle, are new classes of compounds, whereas others, including the 4-aminquinoline ferroquine, are not. These new drugs are in Phase II clinical trials and are unlikely to become available within the next few years for rapid deployment in regions where ART-R is prevalent or emerging [59–61].

Alternative strategies involving the innovative use of existing ACTs have been proposed. Prolonging the duration of therapy, either to 5 days with the same ACT or sequential 3-day treatments with two different ACTs, are likely to be efficacious and well tolerated, but the longer regimen may compromise adherence to the full course [62]. Using multiple ACTs as first-line treatment at the same time in an area or sequential deployment at fixed intervals could reduce the selective pressure on individual partner drugs while sustaining efficacy [63]. Whether under-resourced countries are able to manage these novel strategies effectively remains unclear. Triple artemisinin-based combination therapies (TACTs) provide another promising option that could be rapidly deployed. TACTs are combinations of an artemisinin derivative and two partner drugs selected based on their elimination half-lives.
and observed parasite resistance profiles to ensure mutual protection [60,64,65**,66]. Two such TACTs, artemether-lumefantrine with amodiaquine and dihydroartemisinin-piperaquine with mefloquine have been shown to be well tolerated and highly efficacious in Thailand, Cambodia and Vietnam where dihydroartemisinin-piperaquine failed in nearly 50% of patients [48**]. Another triple combination containing arterolane-piperaquine and mefloquine was also recently found to be safe, well tolerated and efficacious in Kenyan children with uncomplicated falciparum malaria [67]. Finally, administering a single gametocytocidal low dose of primaquine (0.25 mg/kg) along with ACTs or TACTs as is currently recommended by the WHO in low-transmission areas is important to reduce the transmission of multidrug-resistant *P. falciparum* [8].

**Treatment of severe malaria**

Artemisinin resistance is a threat to the life-saving effect of parenteral artesunate in the treatment of severe falciparum malaria. The reduction in case fatality in patients treated with artesunate compared with quinine is in particular prominent in adult and paediatric patients presenting with ring-stage hyperparasitaemia [6,7]. This suggests that the rapid ring-stage parasiticidal effect of artesunate, which is absent with quinine treatment, is important in saving lives. In ART-R *P. falciparum*, artemisinin sensitivity of ring-stage parasites is compromised, which would allow continued parasite maturation to the trophozoite and schizont stages that sequester in the microcirculation of vital organs contributing to organ failure. Case reports from the GMS show dangerously delayed parasite clearance after intravenous artesunate in artemisinin-resistant severe malaria, resulting in death or requiring rescue treatment with quinine [68]. However, a recent observational study from Vietnam did not report a high case fatality after intravenous artesunate treatment in patients with severe malaria acquired in areas of artemisinin resistance [69]. The WHO guidelines on the management of severe malaria suggest a combination of intravenous artesunate and quinine in infections originating from areas with artemisinin resistance [70]. This combination has proven to be well tolerated [71], but evidence from randomized controlled trials of its benefit above treatment with artesunate alone in ART-R severe malaria is currently still lacking.

**FUTURE PERSPECTIVES AND CONCLUSION**

Artemisinin resistance in falciparum malaria causes delayed parasite clearance, and increases reliance on the ACT partner drug to cure the infection. However, more than a decade after its first description, the ART-R phenotype has not evolved into a further increase in the PC½ above approximately 7 h or an extension of resistance to the more mature asexual parasite stages [14,20*]. In the presence of an effective partner drug, ACTs are thus still efficacious. The possibility that other and/or *pfk13*-independent forms of ART-R may emerge cannot be eliminated, and comprehensive and systematic surveillance for ART-R with wide geographic coverage is essential, using molecular or genomic epidemiology to target *in vitro* and/or in vivo phenotypic assessments wherever molecular evidence of ART-R is found.

In the GMS, artemisinin resistance is compounded by partner drug resistance, rendering the infection increasingly difficult to treat. This concern, and the fear of resistant parasites spreading to other malaria endemic regions, in particular sub-Saharan Africa, has prompted a concerted effort to eliminate malaria from the GMS. This effort is now well underway and the number of *P. falciparum* infections has dropped to under 20,000 cases in 2020 [72]. ART-R has to date not spread from the GMS, but has emerged independently in several countries. In particular, the recent reports from Rwanda are concerning, and jeopardises the efficacy of artemether-lumefantrine in the country.

New drugs and strategies to protect existing drugs from falling to resistance will be critical countermeasures against ART-R and multidrug-resistant falciparum malaria. There are several promising drugs in the development pipeline, but none are likely to be available in the next few years. In the interim, judicious use of antimalarials already on the market to prolong their utility by combining them as TACTs or finding practical ways to implement multiple first-line therapies will be important strategies to ensure continued efficacious antimalarial treatment [63,65**,73].

In conclusion, ART-R and ACT partner drug resistance are expanding and constitute a major threat to malaria control and elimination. Close surveillance of drug resistance and implementation of strategies to treat or delay drug-resistant malaria are paramount. Investments to support the systematic and proactive deployment of surveillance and treatment strategies could avert many deaths from multidrug resistant-falciparum malaria in the future.

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