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REVIEW

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Current and emerging strategies to combat antimalarial resistance

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ABSTRACT

Introduction: Since the spread of chloroquine resistance in *Plasmodium falciparum* in the 1960s, recommendations have been made on how to respond to antimalarial resistance. Only with the advent of artemisinin partial resistance were large-scale efforts made in the Greater Mekong Subregion to carry out recommendations in a coordinated and well-funded manner. Independent emergence of parasites partially resistant to artemisinins has now been reported in Rwanda.

Areas covered: We reviewed past recommendations and activities to respond to resistance as well as ongoing research into new ways to stop or delay the spread of resistant parasites.

Expert opinion: Inadequate information limits the options and support for a strong, coordinated response to artemisinin partial resistance in Africa, making better phenotypic and genotypic surveillance a priority. A response to resistance needs to address factors that may have hastened the emergence and could speed the spread, including overuse of drugs and lack of access to quality treatment. New ways to use the existing treatments in response to resistance, such as multiple first-lines, are currently impeded by the limited number of drugs available.

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malaria; drug resistance; Plasmodium falciparum; antimalarial; selection pressure; drug efficacy

1. Introduction

Malaria remains a major public health problem. In 2019, there were an estimated 229 million malaria cases and 409,000 malaria deaths worldwide. The World Health Organization (WHO) African Region, accounted for about 94% of cases. Two malaria species are the most prevalent: *Plasmodium falciparum* and *Plasmodium vivax*. *P. falciparum* causes most cases and deaths; the estimated proportion of cases due to *P. vivax* in 2019 was 3% [1]. Antimalarial compounds are used to treat malaria and protect at-risk populations; having safe and effective treatments prevents malaria patients from developing severe disease and death. Resistance has repeatedly caused the loss of key drugs resulting in increased morbidity and mortality, and the need to continue the search for new drugs.

Drug resistance in *P. falciparum* has been and remains one of the greatest threat to malaria control and elimination [2]. Since the spread of chloroquine resistance in *P. falciparum* in the 1960s, recommendations have been made regarding the best ways to respond to antimalarial resistance with the aim to save the treatments used and prevent or delay drug resistance. However, only with the advent of artemisinin partial resistance were large-scale efforts made in the Greater Mekong Subregion (GMS)[1] to carry out recommendations in a coordinated and well-funded manner. Recently, a change in *P. falciparum* response to artemisinin was detected outside the GMS in Rwanda [3]. Furthermore, the development of resistance to the partner drugs used in the artemisinin-based combination therapies (ACTs) continues to pose a challenge in the treatment of malaria [4]. This article focuses on resistance in *P. falciparum* and reviews the efforts done since the 1960s to respond to antimalarial resistance and outlines the available tools to respond to the challenges currently faced.

2. Resistance in the era of the Global Malaria Eradication Programme

In 1955, the Global Malaria Eradication Programme (GMEP) was approved by the 8th World Health Assembly. The central and often sole intervention planned in GMEP was insecticide residual spraying (IRS) using dichloro-diphenyl-trichloroethane (DDT). Initially, the use of antimalarial was thought only to have an important role in the later stages of the eradication efforts when few cases remained [5].

Concerns over the development of insecticide resistance and lack of progress in some countries led to explorations of ways to use chemotherapy not only in the last stages of eradication but also as an auxiliary to IRS to more rapidly achieve elimination [6,7]. One method tested in several countries was the introduction of cooking salt medicated with an antimalarial drug. Cooking salt with pyrimethamine or chloroquine was tested in several countries including Brazil, Cambodia, Ghana, Guyana, and Uganda. In Brazil, a large-scale trial was done in 1959 covering the entire Amazon region and a population of 2.5 million, supplying cooking salt containing 0.25% chloroquine [6,8–10].

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Article highlights

- Antimalarial resistance continues to be a threat to public health, compromising the ability to prevent and treat malaria, and necessitating a constant search for new drugs.
- In the past, the antimalarial resistance response has been based on a strategy seeking to isolate areas where resistance has been identified.
- However, the ability to effectively respond to resistance has been impeded by lack of information on the spread of resistance.
- The emergence of artemisinin partial resistance in the countries of the Greater Mekong Subregion prompted a coordinated, well-funded response to resistance which has helped significantly lower the malaria burden making malaria elimination a feasible target.
- Artemisinin partial resistance causing delayed clearance after treatment with an artemisinin has been identified in studies in Rwanda and is probably present in other parts of eastern Africa.
- The recommended first-line artemisinin-based combination therapies (ACTs) are still reported to be efficacious. However, if parasites carrying resistance to both artemisinin and ACT partner drugs spread like it happened in the Greater Mekong Subregion, the consequences would be disastrous.
- The emergence of artemisinin partial resistance in Africa requires a response that includes improved phenotypic and genotypic surveillance and addresses factors that may have hastened the emergence and could speed the spread.
- Investments made due to COVID-19 in laboratory infrastructure and training of staff could potentially be leveraged to improve surveillance of antimalarial resistance and efficacy.

Countries in the GMS employed antimalarial drugs as a central part of their eradication program already from early in the eradication efforts. In Myanmar and Thailand, single doses of chloroquine and pyrimethamine were administered to suspected cases encountered during house visits. In Cambodia, routine administration of chloroquine and pyrimethamine at a fixed interval was used as a supplementary measure to IRS in areas with population movement [11]. Pailin province in eastern Cambodia had attracted gem-miners since the late 1940s coming to mine sapphires and rubies. There was a steady influx of nonimmune young adults living often in only rudimentary shelters and in the presence of *Anopheles dirus*, an exophilic vector difficult to control with residual spraying. Mass drug administration (MDA) with pyrimethamine and chloroquine was carried out in Pailin, twice a year from 1955 to 1957, weekly from 1958 to 1959, and indirectly through medicated salt from 1960 to 1962 [12].

2.1. Emergence of resistance

Cases of *P. falciparum* resistance to proguanil and pyrimethamine had been reported in 1948–50. However, prior to the initiation of the GMEP in 1955, significant drug resistance to chloroquine or amodiaquine had not been reported [13]. Nevertheless, already 2 years after the launch of GMEP, in 1957, resistance to chloroquine was first suspected in eastern Thailand due to delays in the clinical response of *P. falciparum* to chloroquine [14]. Further studies in eastern Thailand found falciparum parasites resistant to all widely used drugs except quinine [15]. Chloroquine resistance and pyrimethamine resistance were identified in Pailin in 1962 [12]. In South America, chloroquine resistance was first observed in Columbia in 1959 [16,17]. The widespread use of chloroquine helped make progress in reducing malaria mortality and morbidity. However, the success of chloroquine also helped facilitate the spread of resistance. By 1964, chloroquine resistance had been reported from Brazil, Cambodia, Columbia, Guyana, Malaysia, Thailand, and Vietnam [16,17].

2.2. Early responses to resistance

The prevalent belief that IRS would pave the way for eradication meant that the appearance of reports of resistance to chloroquine, only a few years after the launch of GMEP, was not given the attention warranted. Global technical recommendations in 1960 in response to the potential emergence of chloroquine resistance stressed that efforts to discover resistance early should be made, and when present, a rapid change to a drug of a different chemical class should occur. It was concluded that it seemed 'that combined use of drugs with different types of actions and at adequate dosage will prevent the development of resistance' [8].

Another WHO meeting was held in 1964 to review resistance of malaria parasites to drugs. While there was an understanding of the urgency in improving the knowledge of the distribution of resistance and the need to establish standard criteria of resistance to chloroquine, it was thought that with intelligent use of drugs, selection of parasites less sensitive to chloroquine could be avoided. The meeting formulated the first proposals for countermeasures to be taken when drug resistance had been confirmed. The priority was given to eliminate the resistant parasites so that foci where resistance had emerged did not remain a threat to the fight against malaria, and to plan future case management in areas of resistance in such a way that effective treatments would be available for acute infections. To eliminate the resistant parasites, it was recommended that every foci of resistance should be treated as a separate problem. Effective treatment of confirmed cases should prevent onward transmission. Presumptive treatment done without microscopic confirmation of malaria should only be done with treatment able to cure the patient and render them noninfectious to mosquitoes. Where possible, MDA schemes using a drug to which resistance had developed should switch to another drug. Other measures proposed included extended use of vector control not only in the areas with resistance but also in the adjacent areas to try and provide a barrier that would contain the spread of resistant parasites. Population movement across this barrier should, to the extent possible, be treated as population movement into areas from which malaria had been eliminated [16]. These proposed countermeasures depended on both up-to-date information on the emergence and spread of resistance and the availability of sufficient resources to react as needed. However, the lack of information on the

developing patterns of resistance and limited alternative drug choices impeded effective responses in the field.

There are records of local responses to resistance. In Guyana, chloroquinized salt was used from 1961 to 1965 in areas with sparse population and considerable population movement. This resulted in a reduction in cases, but chloroquine-resistant strains were imported into areas bordering Brazil in 1962. The use of DDT in house spraying in the border areas complemented by similar operations across the border stopped this spread of the resistant strains [9,18,19].

2.3. Aftermath of the eradication programme

The lack of progress and resurgence of malaria in some areas led to the recognition that there were areas where malaria elimination was not feasible in the short term. Consequently, in 1969, the time-limited goal of eradication was effectively abandoned. Resources and support for malaria activities had decreased and diminished further in the following decades. Due to lack of resources, insecticide resistance and some public concerns over the widespread use of DDT, there was a drastic reduction in the use of residual insecticides and chloroquine-resistant strains of *P. falciparum* spread to many areas, including areas where the malaria prevalence had been greatly reduced during the GMEP. Where there were no longer the resources to continue the IRS campaigns or insecticide resistance hampered the usefulness of these, chemotherapeutic measures became the main tool available [5,20]. This was even more so in remote, forested areas. Economic crisis and conflicts led to increased number of people living in such areas, often working in agriculture or mining. Here, the lack of other malaria control measures and the influx of nonimmune people often led to an extensive and unregulated private sector market trade of antimalarial drugs [5,21].

By 1980, chloroquine resistance had spread through much of South America, Southeast Asia, and North-East India [22,23]. Measures to help contain the spread of resistance were included in some of the national malaria plans developed after the end of the GMEP. In India, the National Malaria Eradication Program had seen early success, bringing the number of cases reported in 1961 to below 50,000. After 1965, malaria resurged and by 1975, 6.5 million cases were reported. In response, the Modified Plan of Operation was launched in 1977 with a *P. falciparum* Containment Programme. This program included the establishment of teams in strategic areas for monitoring of two-way movement from areas known to have foci of drug-resistant strains in North-Eastern regions, and to facilitate actions necessary to prevent spread of the resistant strains [24]. However, by 1977 resistance had likely already spread outside the north-western part of India.

The difficulties in making a sustained impact in the intensive malaria transmission in Africa through large-scale DDT-spraying programs meant that chloroquine was distributed in huge quantities during the GMEP. Reports of nonimmune visitors to Kenya and Tanzania getting malaria and not responding to a standard dose of chloroquine appeared in 1978 [25,26]. Through the 1980s and 1990s, chloroquine resistance spread through Africa from east to west. In the 1970s, the death rate from malaria among children in Africa was almost half the level of the pre-chloroquine years, but hospitalizations and deaths rose again with the spread of chloroquine-resistant parasites [27,28].

In 1981, WHO reviewed the proposed activities to be taken toward prevention and containment of *P. falciparum* resistance. These activities were recommended to be targeted in areas defined by the presence and risk of resistance. In the areas with widespread resistance, the emphasis was on mapping resistance, vector control and providing effective treatment for *P. falciparum* based on parasitological diagnosis, establishment of check posts on known migration routes from chloroquine resistance areas, and establishment of systems with the ability to detect and investigate treatment failures [23].

Pyrimethamine monotherapy had been widely used in the 1950s and 1960s as treatment and for mass prophylaxis. Later, pyrimethamine was reformulated and used in combination with sulphadoxine as sulphadoxine-pyrimethamine (SP). SP became the recommended first-line treatment in many countries, where resistance rendered chloroquine unusable [29,30]. Low-level resistance to pyrimethamine emerges easily; genetic profiling of resistant strains showed that higher-level resistance emerged first in Southeast Asia on the Cambodia–Thailand border areas and then spread to Africa [31]. Pyrimethamine resistance was initially masked by sulphadoxine but resistance to sulphadoxine emerged in both Africa and Asia [29,30].

SP became the first-line treatment for falciparum malaria in Thailand in 1973 and SP was widely available in local pharmacies, used for prophylaxis and treatment of fevers not confirmed to be caused by malaria. In the early 1980s, SP efficacy was so low that Thailand changed initially to a 7-day treatment with quinine-tetracycline. In 1985, mefloquine was introduced, initially in combination with sulfadoxine and pyrimethamine, and from 1991 as monotherapy. Mefloquine was restricted for use by the malaria control program and government hospitals only for the treatment of microscopically confirmed falciparum malaria. However, even before the introduction of mefloquine as a first-line treatment, mefloquine resistance was detected on the Cambodia-Thailand border [21]. Mefloquine was widely available in countries neighboring Thailand, where conflicts and high number of migrants often meant that access to organized malaria control measures was limited. Resistance to mefloquine spread, leading Thailand in 1995 to become the first country to introduce a combination treatment with mefloquine and artesunate (an artemisinin derivative, described in the next section). It was first used only in selected areas deemed to be high-level multidrug resistance zones on the border with Myanmar and Cambodia. Initially, it was given as a two-day treatment and from 2007 as a three-day treatment [21,32,33]. In 2000, Cambodia also introduced the combination treatment with mefloquine and artesunate [34]. In 2000 and 2001, WHO first discussed the use of combination therapies and WHO recommended that countries experiencing resistance to monotherapies should adopt an ACT as first-line treatment for

uncomplicated *P. falciparum*. In 2006, this recommendation was expanded to all countries [35–37].

3. Era of artemisinin

Fueled by the spread of resistance to the most widely used antimalarial drugs, Chinese researchers discovered the antimalarial activity of artemisinin in 1972, based on research on the antimalarial properties of medical plants described in ancient texts [38]. The advantages and disadvantages of artemisinin and artemisinin derivatives (such as artesunate) were clear from the beginning. The drugs were well tolerated and fast-acting, and quickly reduced the number of parasites in the blood. However, effective drug concentration levels were only maintained in the plasma for a relatively brief period after drug administration, and short oral treatment courses resulted in high rates of recrudescence. To prevent recurrent parasitemia, 7 days of treatment was needed when using artemisinin or an artemisinin derivative as a monotherapy [39,40]. Combining an artemisinin derivative with a partner drug with a longer half-life into an ACT takes advantage of the rapid action of the artemisinin derivatives, while the partner drug helps prevent recrudescence, even after a short three-day treatment [41].

3.1. Artemisinin resistance containment

At the time ACTs were introduced, there had been no documented resistance to artemisinin and its derivatives, and it was believed that the rapid elimination of artemisinins from the body would help delay if not prevent the development of resistance. However, from 2003, data began to emerge showing prolonged clearance times after treatment with either artesunate plus mefloquine for 3 days or artesunate monotherapy for 7 days in the areas around the Cambodia–Thailand border [33,42]. In efficacy studies, this was typically seen as a higher than expected proportion of patients with parasites in the blood on day 3 after the start of treatment [2]. Initially, the priority was to confirm if these observations could reflect the emergence of genuine resistance. The Bill & Melinda Gates Foundation funded the Artemisinin Resistance Confirmation, Characterization and Containment (ARC3) project coordinated by WHO that supported treatment efficacy trials. Data collected in 2007 and 2008 found that *P. falciparum* had reduced in vivo susceptibility to artesunate in western Cambodia as compared with north-western Thailand. There were discussions as to what term to use to describe the delayed parasite response. Initially, the term 'artemisinin tolerance' rather than resistance was used. More recently, 'partial resistance' has been used to describe parasites that may not clear as fast as fully sensitive parasites but will still be cleared when treated with a 7-day treatment of artesunate. While Pailin was seen as the epicenter, the actual geographical extent of the problem was still not very clear. However, researchers conducting the studies called for urgent containment measures due to fear of resistance spreading to other countries and continents, with potentially catastrophic global consequences [2,33,42–44].

The ARC3 project also sought to identify strategies to contain the spread of artemisinin-resistant malaria within Southeast Asia. Part of this work was to better understand the factors contributing to the development of drug resistance along the Cambodia–Thailand border. One of the factors identified was the large number of migrants and mobile populations in the areas, many of whom had no immunity and often only had access to expensive and poor quality treatments. In Cambodia, the majority of people with fever sought treatment from the unregulated private sector, where artemisinin monotherapies were widely available [33]. Counterfeit artesunate was widespread [45]; these products have obvious potentially dangerous consequences for patients, and their use can contribute to development of drug resistance. Higher prices of artemisinin and the quick resolution of clinical symptoms also lead to people prematurely stopping treatment, contributing to selection pressure and continued infectiousness. The widespread mefloquine resistance in Cambodia and Thailand when artesunate+mefloquine became the recommended treatment could also have played a role in the decline in parasites' response to artemisinin [32,46]. To reduce the selection pressure globally, WHO Member States had adopted the World Health Assembly resolution WHA60.18 in 2007 calling for a progressive removal of oral artemisinin-based monotherapies from markets [47].

In 2008, the artemisinin resistance containment and elimination (ARCE) project was started. This project was like ARC3 funded by the Bill & Melinda Gates Foundation and coordinated by WHO. The goal of the project was to contain artemisinin-tolerant *P. falciparum* parasites by removing selection pressure and reducing and ultimately eliminating falciparum malaria. Implementation of field activities began in May 2009 and the project ran until November 2011. The activities were coordinated through an international task force, as well as Thai and Cambodian National Task Forces [48,49].

Many of the strategies used were those proposed previously in the 1960s, but these had never been implemented to the same degree as was now envisioned in what was defined as containment zones. Zone 1 was defined as the areas around the Cambodia–Thailand border where there was evidence of artemisinin resistant *P. falciparum*. Zone 2 covered areas where there was no evidence of resistance, but the risk was considered high due to the proximity to Zone 1. Approximately 400,000 people were targeted in areas labeled as zone 1 and 4.86 million people were targeted in areas labeled as Zone 2 [49].

Removing artemisinin selection pressure was not possible as safe, efficacious, and affordable alternatives to ACTs were not widely available. However, mathematical modeling indicated that the most effective intervention to eliminate artemisinin-resistant malaria was to ensure a switch of treatment from artemisinin monotherapy to ACTs. As ACTs were more effective against artemisinin-sensitive parasites, the remaining last parasites were likely the most resistant. Thus, any strategy employing artemisinin needed to be sustained until elimination is achieved [44,50]. During the ARCE project, patients detected in zone 1 in Thailand were treated with atovaquone-proguanil as a directly observed treatment. Artesunate-mefloquine (AS-MQ) remained the first-line treatment in Cambodia until 2009, when co-formulated dihydroartemisinin and piperaquine (DHA-PPQ) became the first-line

BOX 1. Specific objectives of the Artemisinin resistance containment and elimination project (ARCE)

1. To eliminate artemisinin-tolerant parasites by detecting all malaria cases in target areas and ensuring effective treatment and gametocyte clearance.
2. To decrease drug pressure for selection of artemisinin-tolerant malaria parasites.
3. To prevent transmission of artemisinin-tolerant malaria parasites by mosquito control and personal protection.
4. To limit the spread of artemisinin tolerant malaria parasites by mobile/migrant populations.
5. To support containment/elimination of artemisinin-tolerant parasites through comprehensive behaviour change communication (BCC), community mobilization and advocacy.
6. To undertake basic and operational research to fill knowledge gaps and ensure that strategies applied are evidence-based.
7. To provide effective management, surveillance and coordination to enable rapid and high-quality implementation of the strategy.

BOX 2. Main elements of the *Global plan for artemisinin resistance containment* (GPARC)

The GPARC sets out a high-level plan of attack to protect ACTs as an effective treatment for *P. falciparum* malaria. The GPARC has two goals:

- contain or eliminate artemisinin resistance where it already exists;
- prevent artemisinin resistance where it has not yet appeared.

The plan makes five recommendations:

- stop the spread of resistant parasites;
- increase monitoring and surveillance to evaluate the threat of artemisinin resistance;
- improve access to diagnostics and rational treatment with ACTs;
- invest in artemisinin resistance-related research;
- motivate action and mobilize resources.

treatment for *P. falciparum* malaria in zone 1. However, the efficacy of DHA-PPQ declined fast, and in 2012, atovaquone-proguanil also became the first-line treatment in Pailin, Cambodia [49].

The ARCE project employed a variety of new tools and technologies to meet the objectives (BOX 1), including ensuring good access to testing and treatment. More than 3000 village volunteer workers were provided with rapid diagnostic tests (RDTs), thereby extending access to testing and treatment in villages. In addition, 128 migrant workers were trained to provide testing and treatment to migrant populations. Mobile phones and online reporting were tested for use for more responsive surveillance [51]. In Cambodia, the market authorization for all oral artemisinin monotherapies was withdrawn in March 2009, and the ban was enforced by regular inspections by 200 law enforcement officers known as the 'Justice Police' [51]. This did affect the availability of artemisinin monotherapy: of private sector outlets stocking antimalarials, the proportion stocking oral artemisinin monotherapy fell from 20.3% in 2009 to 4.2% in 2011 [52].

Originally, a mass screening and treatment was envisioned, but early pilot schemes could not be scaled up due to lack of human resources [53]. Instead, a focal screening and treatment was carried out in Pailin, Cambodia. A total of 6931 individuals were screened using PCR; the prevalence of *P. falciparum* was found to be less than 1%, 96% of the patients were asymptomatic. There was 1.57% prevalence in villages deemed to be high risk based on surveillance data vs. 0.24% for low-risk villages. One village accounted for 50% of all *P. falciparum* cases detected. The findings and the resources required to do the screening and treatment led to researchers making the case in favor of MDA with treatment given to an entire population without prior screening [49,54].

Long-lasting insecticide-treated nets (LLINs) were used for vector control, and 1 net per person was distributed. Distribution of LLINs was only partially successful; the large distribution campaigns resulted in high levels of coverage, but the coverage and usage quickly dropped off due to the highly mobile population. Hammock nets were distributed to mobile populations on both sides of the Cambodia–Thailand border, but coverage among those groups was difficult to measure and maintain. One innovative approach was lending of nets through employers to workers who often stayed at worksites for short periods. Work was also done to have foremen at farms and plantations send sick workers to treatment posts. Furthermore, almost 800 visits were made to do mass screenings of migrant workers or screening of new incomers in collaboration with employers [48,49].

These efforts did have an impressive impact in terms of the overall burden; from 2008 to 2011 reductions in *P. falciparum* cases of 44% to 57% were seen in zones 1 and 2. In the containment zones in Thailand, *P. falciparum* reduced by around 60% over the 2-year period 2009–2011. This reduction was much greater than what has been observed in the country as a whole. However, in Thailand, surveillance showed that reduction in cases was greater in the population of Thai nationality than in the non-Thai population. In the provinces corresponding to zones 1 and 2 in Thailand, the proportion of cases detected among non-Thais were 14.2% in 2009 and 47.4% in 2011 [48,49].

While progress was made toward elimination in the containment zones, as predicted, a higher proportion of the remaining cases showed delayed clearance after treatment with an artemisinin. Based on data on day 3 positivity rates in Thailand, the Thai zone 1 was expanded to cover two full provinces, Trat and Chantaburi [49]. After the end of the ARCE project, containment activities were continued in Thailand and Cambodia funded by the Global Fund to Fight AIDS, TB, and Malaria. In Thailand, the activities were further expanded and

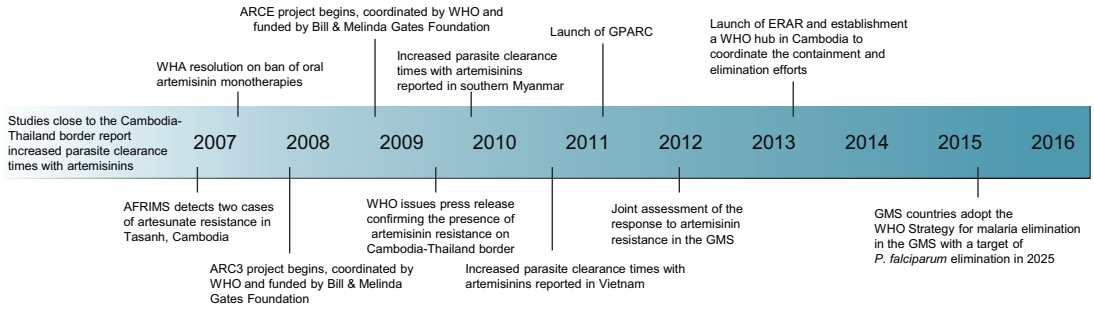

Figure 1. Timeline of events in the emergence and response to artemisinin resistance in the Greater Mekong Subregion.
(AFRIMS: Armed Forces Research Institute of Medical Sciences; ARC3: Artemisinin Resistance Confirmation, Characterization and Containment project; ARCE: Artemisinin Resistance Containment and Elimination project; GPARC: Global Plan for Artemisinin Containment; ERAR: Emergency response to artemisinin resistance in the GMS)

included efforts such as observed treatment for patients and follow-up on day 28 to ensure complete cure.

Learning from the experiences of the containment project, the *Global Plan for Artemisinin Containment* (GPARC) was released in January 2011, having been developed by the WHO Global Malaria Programme through consultation with over 100 malaria experts. The GPARC set out to both contain or eliminate artemisinin resistance where it already existed, and to prevent artemisinin resistance where it had not yet appeared. Five recommendations were formulated to achieve this (BOX 2)

In view of regional differences and varying levels of artemisinin resistance, the GPARC recommendations were to be applied according to an evaluation of level of risk in countries and areas. Different levels of risks were defined as tiers. Areas for which there was credible evidence of artemisinin resistance were defined as 'tier I.' In tier I, an immediate, multifaceted response was recommended to contain or eliminate resistant parasites as quickly as possible. Tier II areas were those with significant inflows of mobile and migrant populations from tier I areas or shared borders with tier I areas. The recommendations for tier II countries were intensified malaria control to reduce transmission and limit the risk of emergence or spread of resistant parasites. In tier III areas, defined as *P. falciparum* endemic areas, which have no evidence of artemisinin resistance and limited contact with tier I areas, prevention and preparedness was focused on scaling up control measures [55].

While containment efforts were ongoing on the Cambodia–Thailand border, in other parts of the GMS, studies were done to collect efficacy data. Evidence started emerging that artemisinin resistance was not only present in the areas defined as zone 1 but also in areas of southern Myanmar bordering Thailand, in Binh Phuoc province in Vietnam bordering Cambodia, and in Yunnan province in China [56]. The areas where resistance was suspected had commonalities: they were typically close to international borders and had high numbers of migrant and mobile populations working in forested areas engaging in, for instance, mining and logging. In Myanmar and Vietnam, plans were developed based on the activities proposed in GPARC and containment activities started in areas where artemisinin resistance was suspected. In Yunnan, malaria elimination was pursued (for timeline, see Figure 1).

In 2011 to 2012, an assessment of the response to artemisinin resistance in the GMS was carried out with the collaboration of WHO, DFID, and USAID/PMI and sponsored by AusAID and the Bill & Melinda Gates Foundation. The joint assessment concluded that a good, if delayed, start had been made to address artemisinin resistance in the GMS. It was found that the approach outlined in GPARC and the associated national level strategies and plans were appropriate but needed modification as new evidence on the nature of artemisinin resistance was produced. Implementation of such a strategy along the Cambodia–Thailand border had significantly reduced the incidence of malaria, especially that caused by *P. falciparum*, and the number of malaria deaths. The joint assessment concluded, however, that 'not enough is yet being done, with enough intensity, coverage and quality, to respond to a problem that could not only slow future progress but also undo the gains already made in malaria control worldwide' [57].

Based on the recommendations from the assessment, the *Emergency response to artemisinin resistance (ERAR) in the Greater Mekong subregion, Regional Framework for Action 2013–2015* was released in April 2013. The framework highlighted key action areas in which progress was urgently needed to contain resistance and move toward elimination of malaria in the GMS. The framework recalled the overarching goal of GPARC in protecting ACTs as an effective treatment for *P. falciparum* malaria. The framework sought to do this by rallying stakeholders to urgently scale-up and increase the effectiveness of interventions to address artemisinin resistance in the GMS [58].

Resistance to partner drugs, as well as to artemisinin, posed a challenge to progress in the GMS. Thailand changed the treatment for *P. falciparum* from AS-MQ to DHA-PPQ in 2015 [59]. However, in Pailin in 2010, just 1 year after DHA-PPQ was introduced, a study recorded a treatment failure rate of 27% (n = 29) [60]. Piperaquine resistance later spread throughout Cambodia, to Lao PDR, Thailand, and Vietnam [4].

Despite some setbacks, the resources invested, and the progress made meant that malaria elimination became within

reach. WHO recommended complete elimination of *P. falciparum* in the GMS based on this progress, along with the reports of resistance to ACT partner drugs and genomic epidemiology studies showing that artemisinin resistance was not only spreading transnationally but also emerging locally at multiple sites [61]. The regional elimination strategy was launched in May 2015 following extensive consultations with countries and partners in the GMS. The ultimate goal of this strategy is to eliminate malaria by 2030 in all GMS countries and, considering the urgent action required against multidrug resistance in the GMS, to eliminate *P. falciparum* by 2025. This goal is within reach; in 2020, 33,781 *P. falciparum* cases were reported from the GMS (see Figure 2).

4. Drug resistance development

Planning measures to prevent and respond to resistance requires an understanding of the causes behind the emergence and spread of resistance and likely scenarios for resistance development. Drug-resistant parasites emerge in two distinct stages: the initial de novo genetic event and a subsequent spread. In vitro studies conducted in the 1990s suggested that *P. falciparum* parasites in Southeast Asia were more readily mutating and developing resistance to structurally and mechanistically unrelated compounds [62]. However, a more recent study of mutation rate variations in Southeast Asian and West African parasites did not find evidence of such hypermutator *P. falciparum* lineages in Southeast Asia. Therefore, other factors that may affect the selection and spread of these mutations must explain the recurring emergences of new drug resistance mutations in Southeast Asia [63].

Drug pressure drives the selection and spread of de novo genetic changes that make a parasite less sensitive to a drug. The selection happens in a 'window of selection' when a drug is present in the blood at levels that inhibit the growth of other sensitive parasites while allowing the nascent resistant parasite to multiply and be transmitted. The window of selection is a function of the half-life of the drug. This half-life is very short for the rapidly eliminated artemisinins but long for the ACT partner drugs [64].

Drug pressure depends not only on the proportion of malaria infections that is treated but also on the overall rate at which people consume antimalarials [65]. In malaria endemic areas, people often have residual drug from previous chemoprophylaxis or treatments. The drug pressure from residual drug probably does not play an important role in the initial de novo selection. The reason is partly numerical; the number of parasites emerging from the liver potentially being exposed to residual drug in a newly infected patient is very low compared with the number of parasites in a hyperparasitemic patient where more than 4% of the red blood cells are infected by malaria parasites [66]. Consequently, treatment failure in a hyperparasitemic patient is the most probable source for the de novo selection [67]. However, residual antimalarial drug is thought to be an important selective force in the spread of resistance when the concentration in the blood prevents new drug-sensitive infections but allows resistant infections to be maintained and transmitted [68].

The risk of resistance spreading and being established in a population is affected by the immunity profile of the community and the local epidemiology [69]. Host immunity kills parasites regardless of drug resistance, so resistance will more easily emerge and spread in a nonimmune population. In low transmission areas with populations with lower acquired immunity, infections are more likely to evolve into clinical diseases requiring treatment, meaning that it is more likely that a resistant parasite will encounter drugs. Furthermore, in high transmission areas, the resistant parasite is more likely to have to compete with a large number of sensitive parasites [67,70].

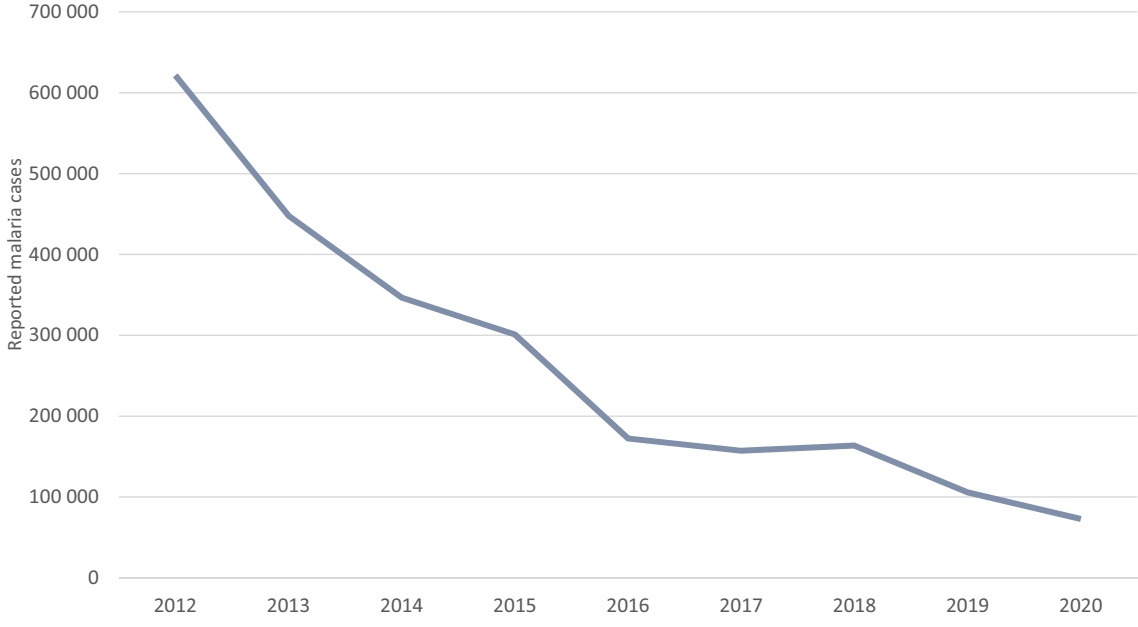

Figure 2. Malaria cases in the six GMS countries[1] (2012–2020) [1].

The survival of resistant parasites is affected by the degree of resistance provided by a given genetic change and the fitness cost associated with this genetic change. Drug resistance mutations often come with a fitness cost giving a disadvantage to the parasite carrying the mutation in the absence of drug pressure, especially in high transmission settings with more frequent intra-host competitions between strains [71,72]. However, continued drug pressure may offer parasites the opportunity to acquire additional mutations that can help compensate for the initial fitness cost. *Plasmodium* reproduces not only asexually but also sexually and the different mutations are less likely to be separated during meiosis and recombination in areas with low transmission, which means that resistance is even more likely to be disseminated in these settings [73–75].

The risk of treatment failure is affected by factors other than parasite resistance and fitness cost, including patient adherence, drug quality, dosing, drug malabsorption, and comorbidities. In controlled trials such as therapeutic efficacy studies, the recommended first-line ACTs are generally found to have high efficacy. Nevertheless, modeling estimated the effectiveness of ACTs in 2016–2019 to be only 71.8% (IQR: 46.9–76.4) largely due to the adjustments applied for drug quality and patient adherence [76]. The consequence is unfortunately that treatment failure is a common occurrence. Just as human behavior affects the likelihood of emergence and selection, it also affects the likelihood of spread between areas through factors, such as migration rates.

Overall, the areas where resistance first emerged have all the hallmarks of resistance breeding grounds – they have been low endemicity areas with an influx of populations with limited immunity, lacking access to quality diagnosis and treatment and with high, unregulated usage of antimalarials.

4.1. Spread or independent emergence

Containment efforts are based on the notion of the development of resistance as rare occurrences where a strain of resistant parasites spread from one area to become prevalent in other areas and threaten the efficacy of first-line treatments. Most of the resistance mutations that in the past have challenged the ability to effectively treat patients have only a few independent origins. Consequently, spread, rather than de novo mutations, appears to be the most important way in which resistant strains are introduced into a parasite population [77].

Chloroquine and pyrimethamine resistance have been used as typical examples of hard selective sweeps where resistant haplotypes spread from a few independent emergences. As noted previously, chloroquine resistance associated with mutations in *Pfcrt* emerged in South America and Asia and spread successfully at least 6 times with resistant strains spreading from Asia to Africa [78,79]. Low-level pyrimethamine resistance associated with the single and double mutations of *Pfdhfr* alleles probably emerged multiple times within Africa in the 1950s and 1960s, but high-level pyrimethamine resistance associated with a triple mutant *Pfdhfr* likely spread from Asia to Africa at the same time as chloroquine resistance in the late 1970s and 1980s [29,80]. Similarly, single mutations in *Pfdhps* conferring low-level resistance to sulfadoxine have been found in many different genetic backgrounds, while parasites with triple mutations appear only to have a few origins [77,81]. Also, mefloquine resistance was first detected in Southeast Asia; in Thailand mefloquine resistance emerged quickly after mefloquine was first introduced in 1985 [21]. However, mefloquine resistance does not appear to have spread west to Myanmar, India, and Africa, probably due to the relatively low mefloquine drug pressure in these areas.

Point mutations in *PfKelch13* have been found to be associated with the delayed parasite clearance after treatment with artemisinins [82]. Analyses have shown that *PfKelch13* mutations were spreading not only in the areas close to the Cambodia–Thailand border but also in western Thailand prior to the start of the artemisinin resistance containment project [83]. At present, at least 135 different *PfKelch13* mutations have been identified in the GMS. Of these, 21 have been shown to be associated with delayed clearance in clinical trials or in vitro [2]. These single point mutations alone have been shown to be enough to affect the clearance rate. However, there are differences in the impact on the clearance rate between the *PfKelch13* mutations, possibly linked to different fitness costs [84].

Pfkelch13 mutations associated with delayed clearance have both spread transnationally and emerged independently within different GMS countries, casting doubt on the potential for successful containment [61]. In parts of the GMS, soft sweeps of different *Pfkelch13* mutations appear to have been replaced with a single hard sweep of a specific *Pfkelch13* mutation, the C580Y [85]. This is partly explained by the rapid spread of a linage carrying both C580Y and genetic changes associated with resistance to piperaquine [86]. *Pfkelch13* mutations have been shown to increase the risk of failure in parasites carrying resistance to piperaquine, as well as in parasites carrying resistance to mefloquine [87,88].

Outside the GMS, *PfKelch13* mutations are frequently identified, but it is rare that these mutations are detected at a prevalence of more than 1% [89]. However, mutations confirmed to be associated with artemisinin partial resistance have been found at higher prevalence in a few locations. In Guyana, *Pfkelch13* mutants were identified in samples collected between 2016 and 2017 among highly mobile patients, most with a recent history of travel in remote gold-mining areas. The mutations were found to have emerged independently rather than having spread from Asia [90]. The mutations have not spread in Guyana at the rates seen in Cambodia, possibly because the mutation was found not only to confer partial resistance to artemisinin but also carry a high fitness cost [91]. Genetic analysis of samples collected in Rwanda between 2013 and 2015 revealed expansion of an indigenous linage carrying a *PfKelch13* mutations, R561H, confirmed to be associated with artemisinin resistance in the GMS [3].

That artemisinin resistance only appears to be spreading in Africa now can have multiple explanations including lower artemisinin drug pressure over the past decades when compared with Southeast Asia, higher immunity,

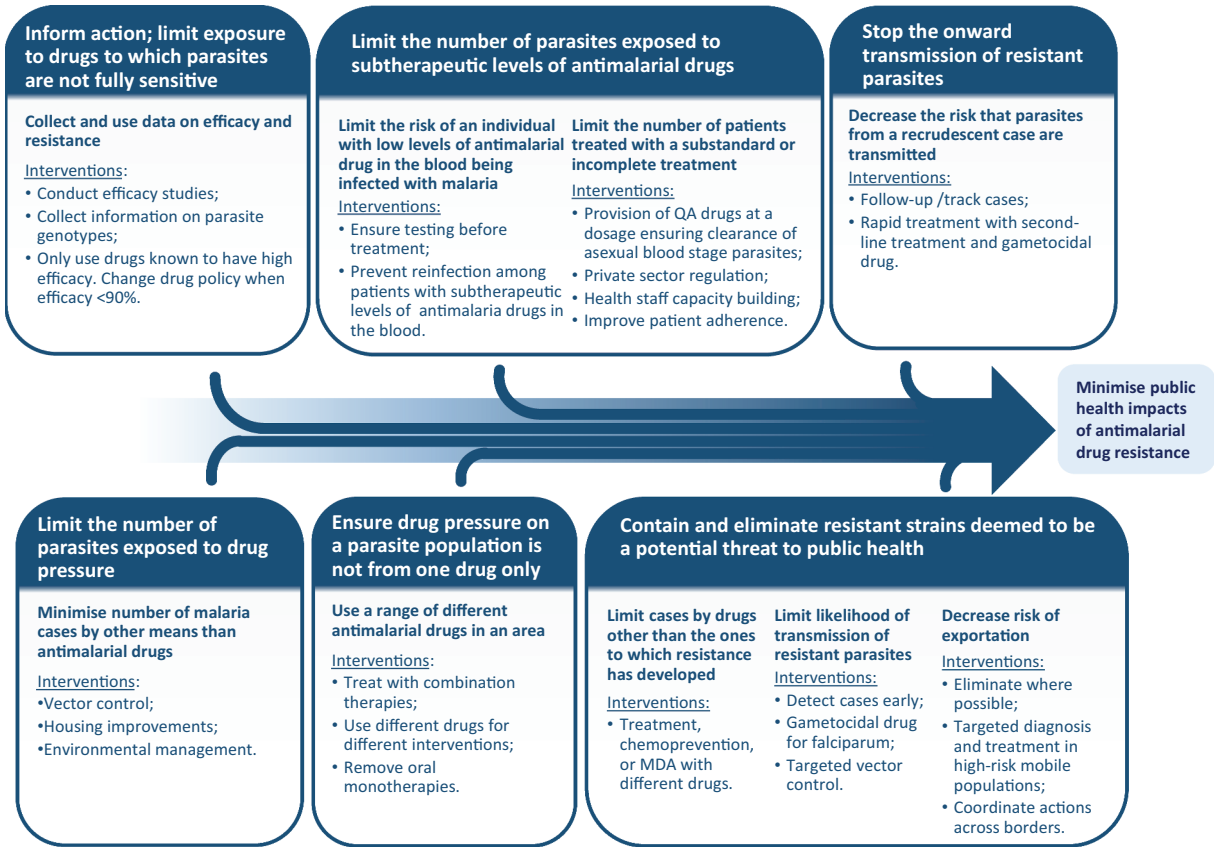

Figure 3. Interventions to prevent and respond to resistance.

and higher transmission level increasing both the competition between parasites and the risk of recombination causing loss of supplementary mutations that could for instance help offset fitness cost. *P. falciparum* strains can vary significantly in their infectivity to *Anopheles* species in vitro. However, available evidence indicates that artemisinin-resistant *P. falciparum* clinical isolates from Cambodia can be transmitted by diverse mosquito vectors of Southeast Asia and Africa [92]. It should be noted that despite the apparent overall differences between the malaria epidemiology in Africa and Southeast Asia, there are lower endemic areas in Africa with an epidemiology more similar to Southeast Asia than to the very high transmission settings in Africa.

Recent developments indicate that the parasite strains that end up posing the biggest risk to the currently used treatments in Africa may emerge there rather than being imported from elsewhere. Nevertheless, it is also possible that what will happen now is multiple soft sweeps of different *Pfkelch13* mutations that are later replaced by a few hard, selective sweeps of haplotypes emerging in low transmission areas. The response to resistance needs to consider these different possible scenarios, seeking both to minimize the impact of the emergence of artemisinin resistance in Africa and the risk of importation of resistant strains from other areas.

Currently, there are no alternatives to ACTs, though the antimalarial drug pipeline is promising [93,94]. The main aim must therefore be to prolong the therapeutic lifespan of the current treatments. The efficacy of ACTs remains high in the areas where delayed parasite clearance after treatment with artemisinins has been detected. The artemisinin component is responsible for most of the short-term clearance of parasites, while the partner drugs are efficacious in the absence of artemisinins and responsible for the overall therapeutic outcome [3]. Consequently, at present, the emergence of artemisinin partial resistance has limited consequences for the patients being treated with ACT as long as the partner drug remains efficacious. The worst scenario would be seeing a pattern similar to what has been seen in the GMS: the emergence of a strain carrying both resistance to artemisinin and to partner drugs.

5. Responding to resistance

Early guidance on the response to resistance recommended treating each focus of resistance as separate problems [16]. The epidemiology of each area differs, as do the main factors driving resistance and the possible responses. In the GMS, the epidemiology, decline in cases, and financial and political support have meant that elimination of *P. falciparum* has been and is a feasible target. However, elimination may not be a feasible target in the short term when resistance emerges in or spreads to higher burden areas. The aim must be to minimize any public health impact where resistance has emerged and limit the risk of spread by activities inside and outside the areas with resistance.

Interventions that decrease the selective advantage of parasites with reduced sensitivity to a drug will slow the

Table 1. Molecular markers of antimalarial drug resistance [2,169] (table adapted based on [2]).

Drug	Molecular markers	
	Gene	Mutation
4-aminoquinolines		
Chloroquine	*Pfcrt*	*K76T + different sets of mutations at other codons (including C72S, M74I, N75E, A220S, Q271E, N326S, I356T, R371I)*
	Pfmdr1 (in combination with *Pfcrt* mutations only)	*N86Y, Y184F, S1034C, N1042D, D1246Y*
Amodiaquine	Yet to be validated	Studies show that amodiaquine selects for *Pfmdr1* mutations
Piperaquine	*Pfpm2-3*	*Pfpm2-3 increased copy number*
	Pfcrt	*T93S, H97Y, F145I, I218F C350R*
Antifolates		
Pyrimethamine	*Pfdhfr*	*N51I, C59R, S108N, I164L*
Sulfadoxine	*Pfdhps*	*S436A/F, A437G, K540E, A581G, A613T/S*
Proguanil	*Pfdhfr*	*A16V, N51I, C59R, S108N, I164L*
Amino-alcohols		
Lumefantrine	Yet to be validated	Studies show that lumefantrine selects for *Pfmdr1* mutations (N86).
Mefloquine	*Pfmdr1*	*Pfmdr1 increased copy number*
Quinine	Yet to be validated	
Mannich base		
Pyronaridine	Yet to be validated	
Naphthoquinone		
Atovaquone	*Pfcytb*	*Y268N/S/C*
Sesquiterpene lactones		
Artemisinin and its derivatives	*PfK13*	List of candidate and validated markers developed

spread of resistance. Consequently, interventions needed include those that decrease drug pressure, ensure that drug pressure on a parasite population is not from one drug only, and that minimizes the risk that parasites from recrudescent cases are transmitted (see Figure 3).

A challenge in every response to resistance has been that the information available has not been sufficiently granular to clearly delineate the spread of resistance. This has hampered the ability to plan and target the response. Lessons learned from the artemisinin resistance containment efforts in the GMS as well as technological advances could help better guide future responses to resistance.

5.1. Surveillance of drug efficacy and resistance

Information on parasite resistance and treatment efficacy has been collected through three main methods: therapeutic efficacy studies (TES), in vitro/ex vivo assays, and molecular markers of drug resistance. WHO recommends that all national malaria programs establish sentinel sites and conduct TES in these sites every 2 years. TES are the gold standard for

informing antimalarial drug policy as outcomes have direct clinical relevance [2]. TES have been a crucial source of information in detecting changes in parasites' response to treatment. Nevertheless, TES are time-consuming and resource heavy. Additionally, the clinical response to treatment depends on factors not related to resistance including immunity. Consequently, detection of changes in parasite genotypes in TES studies may be delayed in highly endemic areas compared to low endemic areas. In vitro and ex vivo assessments of parasites' sensitivity to drugs have the advantage that they are not confounded by host immunity [95]. However, conducting these assessments requires substantial laboratory infrastructure and skilled human resources. Thus, to be able to adequately map the spread of resistance and hopefully detect it before it spreads, phenotypic surveillance needs to be supplemented by genotypic surveillance.

When the response to artemisinin resistance was initiated, there were no known markers of the delayed response to artemisinin. Since then, *PfKelch13* mutations have been identified as molecular markers, and there have been significant advances in sequencing technology. Therefore, mapping the prevalence of *PfKelch13* mutation is feasible and could help national programs to obtain useful information about the extent of resistance and inform appropriate actions. In the past, molecular markers of drug resistance have played a limited role in informing treatment policy. This is partly due to the low predictive value of molecular markers on clinical outcome [96–98]. Nevertheless, molecular surveillance can play an important role in providing early warning of changes happening and, at a minimum, help inform where further phenotypic studies are needed.

Validated molecular markers are not available for key drugs including the ACT partner drugs pyronaridine, lumefantrine, and amodiaquine (Table 1). Advances in sequencing technologies, such as next-generation sequencing (NGS) platforms, allow for targeted deep and whole-genome sequencing and can detect changes in patterns of malaria parasite diversity, potentially providing critical information. Although the costs of NGS technologies have decreased, they still require adequate laboratory infrastructure, expertise in data analysis, and high computing power, not always available in malaria endemic countries. Establishing centers of excellence or regional reference laboratories could help support the overall work. To ensure the accuracy and the comparability of the results from different laboratories, a good external quality assurance system will need to be implemented [99,100]. In some countries, it is possible that investments made due to COVID-19 both in laboratory infrastructure and in training of staff can be leveraged to also strengthen the work for malaria.

Large-scale routine sampling of malaria parasites can be done via existing surveys such as the malaria indicator surveys. Alternatively, dried blood spots or RDTs can be collected from a selection of health centers [101]. One of the challenges in the use of molecular marker data is the time lag to publication, meaning that data is often only publicly available years after the samples have been collected [96,97]. To be of full use for public health, resistance data need to be more readily available and considered part of routine surveillance.

5.2. Reduction of selection pressure

Limited number of drugs means that even when resistance has been detected, halting the use of that drug may not be the best option or even possible. Early guidance recognized that if part of the response requires increased use of the drug to which resistance is developing, then a probable outcome is that the proportion of cases that are resistant will increase. Consequently, seeking to lower transmission and stop the resistance spread with interventions other than drugs must be a basic part of any response to resistance. In the GMS, the usefulness of vector control in some areas has been challenged by the presence of exophagic, early biting mosquitos and transmission taking place in the forest [102,103]. Therefore, efforts have gone into finding new ways of providing protections such as giving forest workers hammock nets, although with mixed success [104–106]. Responses elsewhere will need to be built on an understanding of entomological and human factors contributing to malaria transmission to optimize the use of available tools.

Ensuring that those being treated for malaria are adequately protected by vector control measures could also help lessen the residual drug pressure by limiting the risk that those with low levels of antimalarial drugs in the blood are reinfected with malaria. Previous treatments with antimalarial have been found to be associated with malaria infection [107]; individuals working or living in conditions that once resulted in a malaria infection can be at higher risk of being reinfected.

Other strategies used to seek to reduce the selective drug pressure have been limiting the use of antimalarial drugs to only those who have been confirmed to have malaria by microscopy or RDT. Analyses of data from household surveys in sub-Saharan Africa in 2015–2019 estimates that only 37.7% of children under 5 years with fever who sought care received a finger or heel prick (a surrogate indicator for having been assessed for malaria through RDT or microscopy), while 80.5% received treatment with ACTs [1]. The consequence of an overuse of drugs is increased levels of residual drug pressure. A study in Tanzania in 2015 found that 12.4% of those participating in a cross-sectional survey had lumefantrine or desbutyl-lumefantrine in the blood [108]. The wider availability of RDTs has played a large role in the GMS in making progress toward ensuring that only those with confirmed infection receive antimalarial treatment. The provision of community-based diagnosis and treatment by village malaria workers and community health workers has played a big role in improving access to diagnosis and treatment [109–111] and has been an important component in the response to drug resistance. Combining increased access to quality diagnosis and treatment with training of health staff and information to patients can

help improve treatment adherence and thereby play a role in increasing effectiveness [112,113].

Eliminating substandard medicines, including monotherapies and increasing access to and effectiveness of quality treatment, will minimize the exposure of parasites to subtherapeutic levels of antimalarial drugs. Restricting the availability of substandard drugs and monotherapies requires strengthening the regulatory capacity and quality control laboratories at the national and regional levels [114,115]. A strong regulatory framework needs to be combined with increased capacity to enforce bans. The private sector remains the main source of treatment seeking for febrile illness in many countries. The Affordable Medicine Facility-malaria (AMFm) and Global Fund's Private Sector Co-Payment Mechanism have in some countries improved the quality of treatment provided to patients in the private sector by providing private sector subsidies for quality-assured ACTs, resulting in lower ACT prices and increased availability [116].

5.3. Use combinations of drugs

Ensuring that the drug pressure on a parasite population is not from one drug only can be done both by treating malaria with a combination of different drugs and by using different drugs for different interventions. Interventions currently recommended that do not only use ACTs include chemoprevention strategies such as seasonal malaria chemotherapy (SMC), intermittent preventive treatment in infants (IPTi) and intermittent preventive treatment for pregnant women (IPTp).

5.3.1. Multiple first-line treatments

Currently, countries typically recommend a specific single first-line ACT for the treatment of uncomplicated *P. falciparum*. If efficacy falls below 90%, WHO recommends changing the first-line policy to another ACT with an efficacy above 95% [66]. Having multiple first-line treatments (MFT) has been proposed as a way of delaying the development and spread of resistance and prolonging the useful therapeutic life of the ACTs [117–121]. Most countries have different ACTs registered and permit their use in the private sector. Therefore, where the private sector plays a large role in treatment seeking, different ACTs are commonly used. However, this has not occurred in the context of a planned strategy to prevent resistance by actively promoting heterogeneity of antimalarial drug treatment. The argument in favor of MFL is simple: using different partner drugs will minimize selective drug pressure for a specific drug. However, in 2013, a WHO Technical Expert Group on Drug Resistance and Containment considered the divergent results from mathematical models [117,118] on the benefit of MFT in certain settings, and subsequently refrained from endorsing a general recommendation on the implementation of MFT [122]. MFT strategies were found to delay but not stop the emergence of resistant strains. However, one model found that in areas of high drug usage, MFT performed slightly worse in prolonging the therapeutic life of the used ACTs than did sequential use. Furthermore, inadequate dosage was found to be a much more potent driver of drug resistance than the decision as to whether to deploy drugs as MFL or sequentially [117,118].

Weighing against promoting MFL are both the cost and logistical challenges, as well as the fear that MFL would favor the selection of genotypes resistant to multiple ACT partner drugs earlier than other strategies [118]. In Cambodia, ACTs have been deployed sequentially with DHA-PPQ replacing AS-MQ only to again be replaced by AS-MQ in 2016. This was possible because following the shift to DHA-PPQ, parasites regained susceptibility to mefloquine [123]. In Cambodia, the potential use of MFL is made unfeasible due to the lack of available treatments, and the fear of not having a second-line treatment available to treat failures. At present, the options are limited by the number of ACTs available and by the fact that all the recommended treatments for uncomplicated *P. falciparum* considered for inclusion in an MFL policy contain an artemisinin derivative. There are several new antimalarial compounds in the drug pipeline. When these are ready to be introduced, this must be done in a way to both ensure optimal patient treatment and to maximize the useful therapeutic life, potentially through an MFL policy [121].

5.3.2. Triple combination therapies

The short half-life of artemisinins as one of the two components of the ACTs means that parasites can be exposed to the slowly eliminating partner drug as a monotherapy. Recently, the potential use of triple artemisinin-containing combination antimalarial treatments (TACTs) has been debated [124–132]. TACTs would combine an artemisinin component with two of the currently used ACT partner drugs. The partner drugs most frequently proposed to be combined in TACTs are piperaquine + mefloquine and lumefantrine + amodiaquine due to observations of these drugs having antagonistic resistance mechanisms. Other triple combination therapies that are being tested are an ACT in combination with atovaquone-proguanil [133,134].

A large-scale trial recently evaluated DHA-PPQ, AS-MQ, DHA-PPQ plus mefloquine, artemether–lumefantrine (AL) and AL plus amodiaquine. In countries with piperaquine resistance (Cambodia, Thailand, and Vietnam), the 42-day PCR-corrected efficacy was 48% for DHA-PPQ and 98% for DHA-PPQ plus mefloquine. The trial only has data for AS-MQ in Cambodia, where it was 95%. In Myanmar, piperaquine resistance has not been identified, and the 42-day PCR-corrected efficacy was 100% for DHA-PPQ and 91% for DHA-PPQ plus mefloquine (n = 46). The 42-day PCR-corrected efficacy of AL plus amodiaquine was 98% and similar to AL: 97%; these drugs were tested in Bangladesh, the Democratic Republic of the Congo, Lao PDR, India, and Myanmar [125].

In the GMS, it is not surprising that the addition of mefloquine to DHA-PPQ in areas where parasites are resistant to piperaquine but sensitive to mefloquine results in high efficacy [129]. The main purpose in deploying DHA-PPQ plus mefloquine at present would not be to provide patients with an efficacious treatment, as both AS-MQ and artesunate-pyronaridine remain highly efficacious [2]. Rather, the purpose would be to seek to delay the reemergence of mefloquine resistance and, when it happens, to provide efficacious treatment. However, piperaquine resistance is widespread and parasites carrying resistance to both mefloquine and piperaquine have been identified, meaning that the spread of these

parasites is a risk [135]. Shifting to a triple combination therapies when significant levels of resistance have already been developed to component drugs will mean that the potential benefits of the combination in terms of resistance prevention could be limited [68,130]. Furthermore, while the study reported the TACTs to be safe and well tolerated, adding another drug to established regimens would require further studies on tolerability, toxicity, and drug interactions [131]. The GMS countries aim to eliminate *P. falciparum* by the end of 2023, thus hopefully leaving no need for TACTs or any other new combinations in this region.

A question remaining is the potential usefulness of TACTs outside the GMS. The most likely TACT to be deployed is AL plus amodiaquine as tested in the trial [125]. AL has been observed to select for *Pfmdr1* N86, 184F, and D1246 (the NFD haplotype), while artesunate-amodiaquine (AS-AQ) appears to select for *Pfmdr1* 86Y, Y184, and 1246Y (the YYY haplotype) [136]. The YYY haplotype has been found to be associated with amodiaquine treatment failures in Africa, while the NFD haplotype was associated with AL failures [137]. However, the YYY haplotype is generally not observed in Asia [136]. A study of the efficacy of AS-AQ in Cambodia in 2016 found a high failure rate (19.0%). The parasites were NFD haplotype and amodiaquine resistance was not found to be associated with any of the previously identified molecular markers [138]. The *Pfmdr1* N86Y change has been associated with resistance to both chloroquine and amodiaquine. Countries deploying AL after the withdrawal of chloroquine have seen a return of the wild-type *Pfmdr1* N86 [139]. The same reversal may not happen in countries where AS-AQ is widely used due to the cross-resistance between chloroquine and amodiaquine. It is possible that the opposing selection pressure observed is linked with chloroquine resistance patterns, and the search for good molecular markers for amodiaquine and lumefantrine needs to continue.

Other proposed changes in how the currently available treatments are used include extending ACT regimens from the standard 3 days to 5 or 6 days or using two different ACTs sequentially. An extended 5-day AL regimen was tested in Myanmar [140], and a 6-day AL regime was tested in Tanzania [141] compared with the standard 3-day regime. Both studies found the extended treatment to be safe and well tolerated but not superior to the standard 3-day treatment.

AL and AS-AQ are efficacious in Africa so the purpose of combining the drugs in a TACT or extending the treatment would not be to provide the patients currently being treated with a better treatment but to prevent the emergence and spread of resistance to lumefantrine and amodiaquine. AL and AS-AQ are the most widely recommended first-line treatments in Africa. Losing these drugs before new treatments become available would be devastating. The potential delay in resistance benefiting future patients would have to be balanced with any potentially increased risk for current patients caused by providing an additional drug and drug–drug interaction, and with the resources needed to get co-formulated TACTs with dosing for all age groups or alternative 5 or 6-day regimens ready for and introduced into policy, and the need to allocate more funding to treatment with these drugs versus for instance spending this funding on vector control [126]. Providing combination treatments co-blistered rather than co-formulated could result in more frequent use of one of the drugs alone. Focusing on improving how the currently available treatments and tools are used and developing new classes and combinations of antimalarials rather than spending resources on a temporary solution are more likely to provide the greatest benefit to current and future populations at risk.

5.4. Stopping transmission of resistant parasites

Transmission of malaria from a human is dependent on the presence of the nonpathogenic sexual-stage parasites gametocytes as only gametocytes are infectious to mosquitos. Gametocytes are formed when asexual parasites differentiate into male and female gametocytes. When these are ingested during a blood feeding by a female *Anopheles* mosquito, they are activated in the mosquito midgut into male and female gametes and can fertilize and produce a zygote. This zygote is subsequently transformed into an ookinete, an oocyst, and sporozoites that can be transmitted to humans once again when the mosquito bites and injects saliva [142,143].

Artemisinins are well known for their ability to rapidly kill the asexual parasite in the red blood cells [143]. However, artemisinins have also been shown to be able to block the activation of male gametes, thereby hindering transmission. Some strains carrying *PfKelch13* mutations have been shown to have an increased capability to activate gametes and infect mosquitoes under artemisinin treatment compared with sensitive controls [143]. This could significantly hasten the spread of artemisinin resistance.

Primaquine has been shown to block transmission through its effect on gametocyte persistence and infectivity. Therefore, WHO recommends adding a single low-dose (0.25 mg/kg) of primaquine in combination with ACTs in areas of low transmission or artemisinin-resistant *P. falciparum*. Particularly, areas threatened by artemisinin resistance and areas with elimination programs were expected to benefit from this recommendation [144,145]. Currently, there are no available alternatives to ACTs and the potential role of ACT coverage in driving the spread of *PfKelch13* mutations makes the addition of primaquine in areas of artemisinin resistance a priority.

Treatment failures drive the emergence of resistance by facilitating onward transmission of parasites that have been exposed to drugs [67]. Having systems that follow-up, catch and treat all failures is difficult even in high resource setting. Greater emphasis on identifying treatment failures in the training of staff and in the development of surveillance systems could increase the number of such failures who then receive curative re-treatment before onward transmission. Other options to explore include planning future treatments, so they are not only efficacious in trial setting where adherence is assured; this could be done by reducing the duration of the regimens, ideally to a single dose [146].

5.5. Containing and eliminating resistant strains

Additional interventions proposed in areas where resistance has developed include MDA, which seeks to reduce the number of malaria cases using drugs other than the ones to which resistance has developed. In 2010, an expert meeting was convened to evaluate the appropriateness of including MDA in the strategy to contain artemisinin-resistant parasites in the GMS [147]. The meeting recommended piloting of MDA; this was thought likely to bring about significant reductions in parasite biomass that would diminish the probability that resistant parasites would spread, but MDA was thought unlikely to permanently interrupt *P. falciparum* transmission. The recommended drug of choice was atovaquone-proguanil [147]. Where drugs other than the drug to which resistance has developed are available for the MDA, the rationale is clear: MDA could potentially both lower the malaria burden and the proportion of resistant parasites. The expert group did not endorse the use of ACTs for MDA in the GMS, since the treatment would likely be more effective at targeting artemisinin-sensitive parasites, leading to an increase in the proportion of resistant infections; failure to remove all resistant parasites would eventually allow them to repopulate. No pilot using atovaquone-proguanil was undertaken due to the emergence of a single point mutation in the *cytochrome b* gene conferring high-level atovaquone resistance, which occurred after brief use of this drug as a first-line treatment in parts of Cambodia [49]. Some modeling and reviews argued for the use of MDA with ACTs [148,149], based on the premise that MDA would clear infections among individuals with low-density, blood-stage parasitemia often not detected by RDTs or microscopy. These individuals would not receive treatment when presenting at a health facility with symptoms, would not be detected in any focal or mass screenings, and thus could potentially serve as an infectious reservoir [150–152].

DHA-PPQ was the ACT proposed for MDA due to the long half-life of piperaquine allowing the drug to act as chemoprophylaxis for longer than other partner drugs. It was reasoned that provided full treatment were given, three rounds of MDA would be unlikely to contribute to artemisinin resistance because most individuals would not be hyperparasitemic but rather have low density asymptomatic infections, suggesting that the likelihood of MDA causing de novo emergence would be very low. Furthermore, the short half-life of dihydroartemisinin would provide a too short window of selection for the MDA with DHA-PPQ to drive the spread of artemisinin resistance [148,149].

In a 2015 WHO recommendation on MDA, it was stated that 'Given the threat of multidrug resistance and the WHO call for malaria elimination in the GMS, MDA may be considered as a component of accelerated malaria elimination efforts in areas of the GMS with good access to treatment, vector control and surveillance.' [153]. The recommendation stated that MDA should only be started if there is a good chance that elimination is feasible in the area where it is being administered [153]. Studies were undertaken to assess the effectiveness of MDA with DHA-PPQ in reducing *P. falciparum* incidence and prevalence [53,154–158]. A cluster randomized trial in Myanmar, Vietnam, Cambodia, and the Lao PDR established vector control and community-based case management and provided three monthly rounds of DHA-PPQ MDA in eight villages, while another eight villages served as controls for 12 months. A low dose of primaquine was given on day 1 in all countries except Cambodia. Of the villagers, 87% completed at least 1 round and 57% participated in all 3 rounds. The clearance rate was 87% in Vietnam, 88% in Cambodia, and 100% in Lao PDR and Myanmar.

While the intervention had a substantial impact on the prevalence of *P. falciparum* infections at 3 months, after 12 months, *P. falciparum* infections had returned due to the spread of the remaining infections as well as reintroduction from surrounding areas. The prevalence at 12 months was below baseline levels, and the researchers concluded that MDA might be a useful tool to accelerate falciparum malaria elimination in low endemicity settings. MDA was found to be less effective for *P. vivax* [158].

Modeling was done to compare findings from four established models on the effectiveness of MDA in different settings. It was concluded that while MDA could reduce transmission for a limited time, it has to be repeated regularly for sustained effect [159]. Effective vector control, early diagnosis and treatment, and good surveillance and response systems are prerequisites to achieve and sustain elimination, as well as being a requirement to maintain any reductions gained from an MDA [160]. The potential gain from an MDA needs to be weighed against the considerable resources needed and the potential impact on drug resistance [161]. In large-scale MDA, identifying individuals in whom the treatment fails to clear parasites will be difficult; if the failure to clear parasites is due to partner drug resistance, a large proportion of the remaining parasites will carry this resistance. If the treatment blocks the activation of male gametes only for parasites not carrying mutations conferring artemisinin resistance, successful transmissions are more likely caused by artemisinin-resistant parasites; therefore, primaquine needs to be included [143,145]. Studies are underway on the impact of adding an endectocide treatment, such as ivermectin, to an MDA with an ACT. Endectocides reduce the longevity of *Anopheles* mosquitoes that feed on treated hosts, potentially decreasing transmission and further increasing the impact of the MDA and decreasing the risk of selection of resistant parasites [162,163]. However, with the currently available drugs there are relatively few situations where MDA may play a role in the response to resistance; these will primarily be in isolated, low-transmission settings. Targeted MDA in forest work camps, and other drug-based interventions targeting high-risk groups such as intermittent preventive treatment can play a role in helping to achieve elimination in the GMS as it has proven difficult to reach these population groups [164,165].

In the past, resistance has developed in border areas. Additionally, cross-border migrants often work in areas that put them at high risk of malaria. International coordination and efforts to minimize the barriers to malaria

services faced by migrants are therefore often needed. While artemisinin partial resistance has emerged in multiple locations, the risk of spreading highly resistant strains across continents should not be ignored. In the GMS, actions taken to minimize the risk that resistant strain spreads include attempts to screen migrants and other travelers for malaria and provide them with treatment. A key challenge is that borders are often porous and many of those at highest risk of malaria cross via unofficial routes into neighboring countries [166]. Nevertheless, reducing the risk of spread must be a priority where resistance emerges that is deemed to be a potential threat to public health and a significant number of possible carriers of resistant parasites can be reached. In Cambodia, protocols were developed to screen UN peacekeepers being sent from Cambodia to highly endemic areas in Africa to ensure that resistant parasites were not spread this way.

6. Expert opinion

There is an urgent need to improve the surveillance of resistance. Responses to resistance have built on the idea of treating each focus of resistance as a separate problem; the aim has been to eliminate malaria, where resistance is detected, and limit the number of parasites crossing from the resistant areas to other endemic areas. However, the information available has not been sufficiently granular, and resistance has become discernible too late for containment to be possible.

Data from Rwanda on the emergence of indigenous *PfKelch13* mutations have recently been published from studies starting in 2013. There are indications that *PfKelch13* mutations are spreading elsewhere in Africa. Inadequate information on how widespread these mutations are limits the options and support for a strong, coordinated response. Thus, better phenotypic and genotypic surveillance is a priority. Advances in sequencing technologies and investments made due to COVID-19 both in laboratory infrastructure and in training of staff should be leveraged to also strengthen the work for malaria.

Lack of data should not be used as an excuse for inaction. In the GMS, rapid spread of strains carrying resistance to both piperaquine and artemisinin resulted in DHA-PPQ efficacy falling quickly. The same must not be allowed to happen in Africa; if declining efficacy is observed for an ACT, the ACT needs to be changed rapidly. Where data have confirmed the high prevalence of *PfKelch13* mutations, priority actions include adding a single, low-dose primaquine to ACT treatments. Other activities must be planned based on analyses of key factors that can help explain why resistance emerged where it did and what may cause it to worsen and spread. These activities are likely to include addressing gaps in vector control coverage, and stopping the continued availability of monotherapies and substandard drugs, while at the same time increasing access to early diagnosis and treatment with quality-assured ACTs.

The utility of triple combination treatments based on currently available drugs is affected by both resistance limiting the drug choices and the need for further studies on tolerability, toxicity, and drug interactions before any co-formulated triple combination therapies can become available. Currently, all the possible first-line treatments contain an artemisinin and almost all countries in Africa recommend one of two different ACTs as first-line treatments: AL or AS-AQ. The result is a high degree of vulnerability to resistance to amodiaquine and lumefantrine. Experiences from ACTs in the GMS and TACTs should be used to better combine and make use of current and new compounds to lessen this vulnerability.

Drugs for which resistance has developed have only rarely been used as part of an aggressive strategy to lower transmission. The fear is that doing so would result in a loss of efficacy of the drugs needed to treat patients and that, unless elimination is achieved, there would be an eventual increase in cases and spread caused by parasites with a high level of resistance. At present, MDA can only play a minor role in the response to resistance; this role is limited to isolated low-endemic areas or possibly as a targeted intervention for high-risk groups.

The biggest risk to the currently used treatments in Africa may emerge there rather than being imported from elsewhere. However, the possibility of history repeating itself and parasites with a high level of resistance being imported from elsewhere cannot be ignored. The current efforts to eliminate malaria in the GMS need to continue.

7. Five-year view

Over the last decades, activities and research have focused on how best to respond to resistance in the Southeast Asian context. The progress made toward malaria elimination in the GMS and the emergence of artemisinin partial resistance in Africa means that the focus and funding need to shift. At present, the recommended ACTs are still efficacious in Africa, but resistance to key ACT partner drugs is likely to emerge, and it is possible that artemisinin partial resistance will help fuel the spread of partner drug resistance.

The response to resistance in Africa will to a large extent have to focus on surveillance and getting the basics right: providing access to vector control, diagnosis, and the recommended combination treatments, and eliminating oral artemisinin monotherapies and substandard treatments. If sufficient investments are made, technological advances could mean that the information available in 5 years will be able to better guide the response and improve the use of the tools currently available as well as new tools, such as the vaccine being piloted in young African children. New ways to use the available treatments will continue to be debated, but any benefits of new combination treatments and strategies like multiple first-lines are likely only to be fully realized once new drug compounds are available [167,168].

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1. Covers Cambodia, China (Yunnan province), Lao PDR, Myanmar, Thailand, and Vietnam.

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•• **This is an exhaustive review of our current knowledge on antimalarial drug resistance and mechanism of resistance documented with tables.**