



# Artemisinin resistance and artemisinin-based combination therapy efficacy

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STATUS REPORT

## KEY MESSAGES

1. Artemisinin<sup>1</sup> resistance is defined as delayed parasite clearance following treatment with an artesunate monotherapy or with an artemisinin-based combination therapy (ACT). This represents partial resistance.
2. Delayed parasite clearance does not necessarily lead to treatment failure. In the Greater Mekong Subregion (GMS), high treatment failure rates following treatment with an ACT have almost always been observed in areas where there is concomitant partial resistance to artemisinin and resistance to the ACT partner drug. Outside the GMS, treatment failure with ACTs (artesunate-amodiaquine and artesunate-sulfadoxine-pyrimethamine) has occurred in the absence of artemisinin partial resistance mainly due to partner drug resistance.
3. A molecular marker for artemisinin resistance has been identified and is helping to improve the global surveillance of artemisinin partial resistance.
4. The independent emergence of artemisinin partial resistance in multiple locations in the GMS and the emergence of multidrug resistance, including partial artemisinin resistance and partner drug resistance causing ACT failure, and have led WHO to recommend the elimination of malaria in this region.

## BACKGROUND ON ANTIMALARIAL TREATMENT

Artemisinin-based combination therapies (ACTs) are recommended by WHO as the first- and second-line treatment for uncomplicated *P. falciparum* malaria as well as for chloroquine-resistant *P. vivax* malaria. ACTs combine an artemisinin derivative with a partner drug. The role of the artemisinin compound is to reduce the number of parasites during the first three days of treatment (reduction of parasite biomass), while the role of the partner drug is to eliminate the remaining parasites (cure).

WHO currently recommends five different ACTs. However, WHO is considering the use of artesunate-pyronaridine, a new ACT that has received a positive scientific opinion from the European Medicines Agency (EMA), in areas where other ACTs are failing. In the absence of resistance, all six partner drugs would be highly efficacious as monotherapies at the dose used in the ACT. Two injectable treatments, artesunate and artemether, are recommended for the treatment of severe malaria and should be followed by an ACT once the patient can tolerate oral therapy.

## BACKGROUND ON ANTIMALARIAL DRUG RESISTANCE

### Definitions

Antimalarial resistance and treatment failure can be defined as follows:

- Antimalarial resistance is defined as the ability of a parasite strain to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject;
- Multidrug resistance is resistance to more than two antimalarial compounds of different chemical classes. This term usually refers to *P. falciparum* resistance to chloroquine, sulfadoxine-pyrimethamine, and a third antimalarial compound;
- Treatment failure is defined as the inability to clear malarial parasitaemia or prevent recrudescence after administration of an antimalarial medicine, regardless of whether clinical symptoms are resolved. Many factors can contribute to treatment failure, including incorrect dosage, poor patient compliance, poor drug quality, and drug interactions and resistance. Most of these factors are addressed by therapeutic efficacy studies (TESs).

### Definition of artemisinin partial resistance

Artemisinin resistance is defined as delayed parasite clearance; it represents a partial resistance that has affected only ring-stage parasites thus far. Nevertheless, the majority of patients who have delayed parasite clearance are still able to clear their infections following treatment with an ACT with an effective partner drug or with an artesunate treatment lasting seven days.

In 2014, a molecular marker for artemisinin resistance was identified: Several mutations in the *PfKelch13* (K13) propeller domain were found to be associated with delayed parasite clearance in vitro and in vivo. The identification of the K13 mutations

as markers for artemisinin resistance has allowed for a more refined definition of partial artemisinin resistance that includes information on the genotype.

Not all of the non-synonymous propeller-region K13 mutants reported indicate the emergence of artemisinin resistance; rather, such mutants can represent 'passer-by' genotypes in the absence of evidence for the selection of the mutant K13 genotype. In addition, different K13 mutations have varying effects on the clearance phenotype. The validation of a K13 mutation as a marker for artemisinin resistance requires that a) the mutation has been correlated with slow clearance in clinical studies, and b) the K13 mutation has been correlated with reduced in vitro drug sensitivity (e.g., ring-stage assay – RSA0–3h) using fresh isolates, or reduced in vitro sensitivity resulting from the insertion of the K13 mutant in transfection studies. If a K13 mutation has only been shown to be correlated with delayed parasite clearance during clinical trials but not validated by in vitro data, it is labelled a candidate/associated marker. A current list of candidate/associated and validated K13 propeller mutations can be found in Table 1 (this list will be updated regularly).<sup>2</sup>

TABLE 1  
**Candidate and validated resistance mutations in the K13 BTB/POZ and propeller domain**

VALIDATED		CANDIDATES/ASSOCIATED	
<b>F446I</b>	<b>P553L</b>	P441L	G538V
<b>N458Y</b>	<b>R561H</b>	G449A	V568G
<b>M476I</b>	<b>C580Y</b>	C469F	P574L
<b>Y493H</b>		A481V	F673I
<b>R539T</b>		P527H	A675V
<b>I543T</b>		N537I	

Outside the propeller domain two mutations were reported frequently in clinical studies: K189T and E252Q. Though presence of E252Q is associated with delayed clearance transfection studies did not confirm in vitro resistance. For A578S please see below.

Other less frequent variants were reported to be associated with delayed clearance but without statistical significance due to the low number of cases: D452E, C469Y, K479I, R515K, S522C, N537D, R575K, M579I, D584V, P667T, H719N.

## Consequences

Possible future consequences of slow parasite clearance, or partial resistance, include: a) the development of total artemisinin resistance; b) the loss of artemisinin as an effective treatment for severe malaria; and c) increased de novo resistance to the partner drug, particularly in patients with high parasitaemia at admission, and/or greater selection of partner drug resistance. If resistance to partner drugs increases, treatment failures are likely to increase in parallel.

Nevertheless, for the time being, the majority of patients with delayed parasite clearance can still be cured using ACTs, as long as the partner drug remains effective. There is no evidence that higher levels of artemisinin resistance (full resistance) have emerged. To date, artemisinin partial resistance in the GMS has not been associated with increased morbidity or mortality. Finally, new evidence in the GMS shows that artemisinin did not facilitate the emergence of resistance to mefloquine or piperazine.

## RESPONDING TO DECLINES IN DRUG EFFICACY

### Monitoring the therapeutic efficacy of ACTs

TESs assessing clinical and parasitological outcomes are the main reference from which national malaria programmes (NMPs) determine their national malarial treatment policy. To ensure that the treatments recommended in the national treatment policy are efficacious, WHO recommends that malaria-endemic countries perform routine monitoring of antimalarial drug efficacy at sentinel sites at least once every 24 months in order to detect changes in therapeutic efficacy. Regions for which there is evidence of resistance should consider adding more sentinel sites to facilitate the early detection of additional resistance foci.

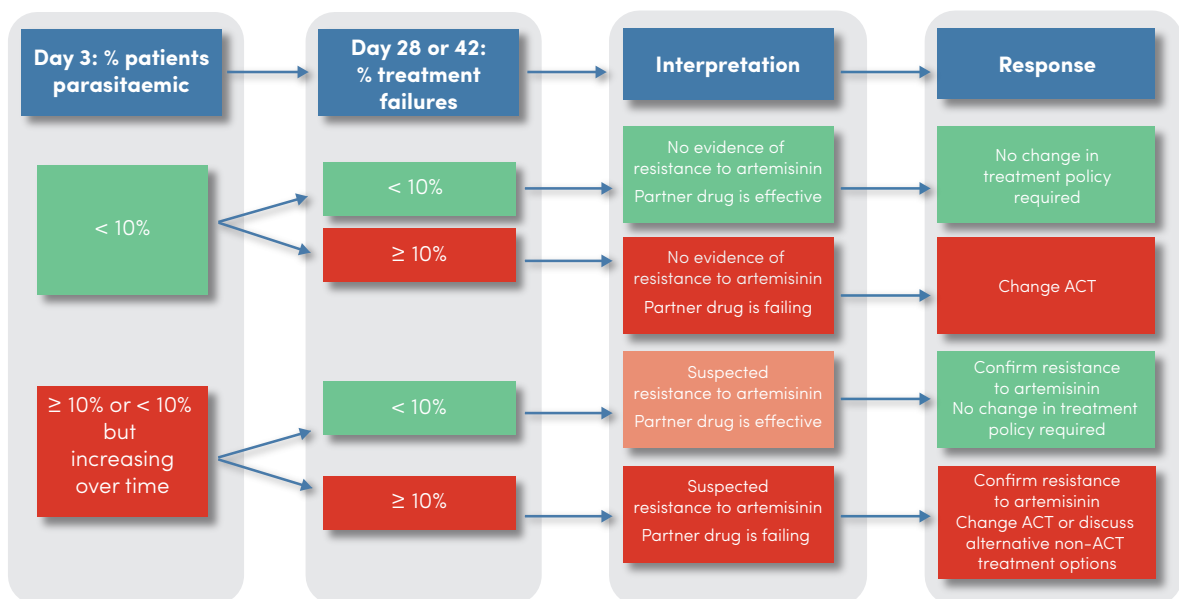
### Changing the treatment policy for *P. falciparum*

Nearly all malaria-endemic countries recommend ACTs for the treatment of uncomplicated *P. falciparum*. TES results for ACTs used in the treatment of *P. falciparum* allow for the determination of:

- the **proportion of patients who are parasitaemic on day 3**, which is currently the indicator of choice for routine monitoring to identify suspected artemisinin partial resistance in *P. falciparum*;
- the **proportion of treatment failure by day 28 or 42** (days of follow-up is determined according to the half-life of the ACT partner drug).

A change in the national malaria treatment policy should be initiated if the total treatment failure rate is  $\geq 10\%$ , as assessed through TESs. NMPs should adopt antimalarial medicines with a parasitological cure rate greater than 95%. Fig. 1 outlines the recommended steps for making treatment policy decisions in response to TES findings.

FIGURE 1  
Decision-making process based on TES results



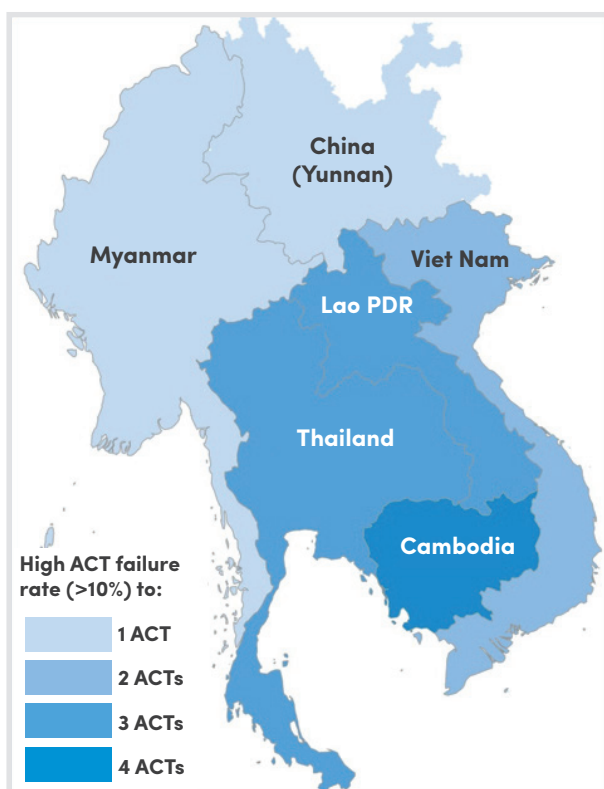
If artemisinin resistance is suspected due to the observation of slow clearance in a clinical trial or TES, K13 marker analysis should be prioritized, e.g., from filter paper blood spots. If resistance is suspected based on a survey with molecular data only, resistance should be confirmed by obtaining information on both the clinical phenotype (delayed parasite clearance) and the K13 genotype from the same parasite strain.

## Elimination of multidrug-resistant malaria: the special case of the Greater Mekong Subregion

The GMS has long been the epicentre of antimalarial drug resistance. Following the initial detection of artemisinin partial resistance in the GMS, containment efforts were initiated to stop the spread of resistant parasites through a comprehensive response combining malaria control and elimination interventions. In April 2013, WHO launched the *Emergency response to artemisinin resistance (ERAR) in the Greater Mekong Subregion: regional framework for action 2013–2015 (1)*.

While the containment efforts were underway, molecular studies found that artemisinin resistance had emerged independently in many areas of the GMS. In addition, resistance had emerged to ACT partner drugs (Fig. 2), threatening the progress achieved in the region to date.

FIGURE 2  
**Numbers of ACTs failing in the Greater Mekong Subregion**



There are currently five ACTs recommended by WHO: artemether-lumefantrine, artesunate-amodiaquine, artesunate-mefloquine, artesunate-sulfadoxine-pyrimethamine and dihydroartemisinin-piperaquine. A sixth ACT, artesunate-pyronaridine, has been given a positive scientific opinion by the EMA under article 58 and is being considered by WHO in areas where other ACTs are failing. By default, artesunate-pyrimethamine-sulfadoxine is considered to have a high failure rate in the region given that high treatment failure rates with sulfadoxine-pyrimethamine and/or quadruple and quintuple *Pfdhfr* and *Pfdhps* mutations (which are usually fixed) have been reported in the region.

Over the last 10 years, the incidence of malaria in the GMS has been greatly reduced. A 2014 analysis considered elimination in the GMS to be technically feasible at a reasonable cost. Therefore, in September 2014, WHO's Malaria Policy Advisory Committee recommended that the goal to eliminate *P. falciparum* in the GMS by 2030 be adopted.

During the World Health Assembly in May 2015, WHO launched a *Strategy for malaria elimination in the Greater Mekong Subregion (2015–2030)* (2), which was endorsed by all the GMS countries. All countries now have national malaria elimination strategies, and the Global Fund to Fight AIDS, Tuberculosis and Malaria has allocated US\$ 242 million to support the countries' move towards malaria elimination. At a malaria elimination side event during the 71st World Health Assembly in 2018, Ministers of Health and other senior representatives from the six GMS countries – Cambodia, China, Lao PDR, Myanmar, Thailand and Viet Nam – signed a Ministerial Call for Action to Eliminate Malaria in the GMS before 2030.

## COUNTRY UPDATES ON ARTEMISININ PARTIAL RESISTANCE

To date, around 200 non-synonymous mutations in the K13 gene have been reported.

Distinct alleles originating from multiple independent events of emergence have been observed in South-East Asia. The surveillance of parasite genotypes, in particular the KARMA project to map artemisinin resistance, has yielded evidence of two distinct epidemiological regions in the GMS: western GMS consisting of China (Yunnan province), Myanmar and western Thailand bordering Myanmar; and eastern GMS consisting of Cambodia, Lao PDR, Viet Nam and eastern Thailand bordering Cambodia and Lao PDR. Five different mutants have been found to have the highest prevalence: F446I, R539T, I543T, P574L and C580Y. Certain mutations have only been found in a particular region. For example, the I543T mutation has only been detected in eastern GMS, whereas F446I has only been detected in western GMS. The F446I mutant appears to be associated with an intermediate rate of delayed clearances.

Currently, the C580Y mutation has been found in several genetic backgrounds (haplotypes) throughout the GMS. The frequencies of different K13 C580Y haplotypes vary by region, and no single haplotype is dominant throughout the GMS. The prevalence of one specific K13 C580Y haplotype has been increasing and replacing other haplotypes in eastern GMS. This indicates a selective sweep in this part of the GMS. The C580Y mutation appears to have now reached fixation in areas of Cambodia where almost all resistant parasites are found to have this specific K13 mutation. The C580Y mutation has been found at a prevalence of up to 70% at the border between Thailand and Myanmar; however, the mutation does not appear to have reached fixation as reported in parts of eastern GMS.

Studies have shown that the predominant K13 mutants found in Myanmar do not appear to have spread from Cambodia, but likely arose independently. K13 mutations remain rare or unrelated to partial artemisinin resistance in Bangladesh and north-east India.

C580Y haplotypes have also been reported in Equatorial Guinea and Ghana, in Chinese travelers returning to their country. Although no investigation into the origin of the mutated parasites has been performed, these mutations most probably emerged

in Africa. Similarly, C580Y mutations have been found in samples from Papua New Guinea and Guyana in what are believed to be local strains. None of these four countries have reported treatment failures linked to C580Y with an ACT.

In Africa, non-synonymous mutations are still rare and highly diverse. Non-synonymous K13 mutations have been reported in Angola, Benin, Burkina Faso, Cameroon, Central African Republic, Comoros, Congo, Côte d'Ivoire, Democratic Republic of the Congo, Equatorial Guinea, Eritrea, Ethiopia, Gabon, Gambia, Kenya, Liberia, Madagascar, Malawi, Mali, Mozambique, Niger, Nigeria, Rwanda, Senegal, Somalia, Sudan, Sierra Leone, Tanzania, Togo, Uganda, Zimbabwe and Zambia. The most frequent allele observed in Africa has been A578S, although it has not been associated with clinical or in vitro resistance to artemisinin. A number of mutations, including some associated with delayed clearance in the GMS (in particular C580Y), have been reported in Africa. However, many of the mutations reported in Africa have not yet expanded in the African parasite populations but careful surveillance is warranted.

## COUNTRY UPDATES ON ACT EFFICACY

Data from the most recent TESs are summarized in tables accessible at [http://www.who.int/malaria/areas/drug\\_resistance/drug\\_efficacy\\_database/en/](http://www.who.int/malaria/areas/drug_resistance/drug_efficacy_database/en/). The summary tables provide treatment failure rates grouped by treatment and country.

### GMS

#### Cambodia

Artemisinin partial resistance was first reported in clinical studies in Cambodia in 2008; however, the retrospective analysis of molecular markers has indicated that artemisinin partial resistance likely emerged prior to 2001 and the widespread deployment of ACTs. Due to the high failure rates associated with artesunate-mefloquine, the first-line treatment for uncomplicated falciparum malaria was changed from co-blistered artesunate-mefloquine to fixed-dose dihydroartemisinin-piperaquine in Pailin in 2008, and then nationwide in 2010. Not long after the implementation of the new treatment policy, an increase in treatment failures after treatment with dihydroartemisinin-piperaquine was observed during TESs. Artesunate-mefloquine was reintroduced as first-line treatment in 2014, but full country coverage was only reached in August 2017. Of the 12 studies on artesunate-mefloquine conducted between 2014 and 2018, all reported efficacy >98%, even though around 95% of parasites were found to carry the K13 C580Y mutation. The proportion of falciparum strains with multiple *Pfmdr1* copy numbers (which confers mefloquine resistance) is currently minimal in the country. Although rare, the existence of parasites with multiple copies of both *Pfplasmepsin 2-3* (the marker for piperaquine resistance in the GMS) and *Pfmdr1* is worrying. Recent TESs with artesunate-amodiaquine have reported high treatment failure rates (14–23%). Artesunate-pyronaridine has recently been found to be highly efficacious in western Cambodia (>95%), contrary to the studies conducted in 2015 in the eastern part of the country.

#### Lao PDR

In Lao PDR, three trials conducted between 2013 and 2017 using artemether-lumefantrine reported failure rates of 10–17.2%. However, the sample sizes for these studies were small. The efficacy of dihydroartemisinin-piperaquine was also

monitored to evaluate its candidacy as a new national malaria treatment policy; however, the treatment failure rate exceeded 15% (though the sample size was also small). Lao PDR plan to monitor the efficacy of artesunate-pyronaridine and artesunate-mefloquine later in 2018.

### **Myanmar**

Artemisinin partial resistance likely emerged along the border between Thailand and Myanmar in 2001, but was only clearly identified in 2008. Since 2009, available data have shown that parasite clearance times are consistently delayed in a significant proportion of patients treated with ACTs. Delayed clearance has been observed with all three first-line ACTs (artemether-lumefantrine, artesunate-mefloquine and dihydroartemisinin-piperaquine) used in Myanmar, yet all three remain efficacious with high cure rates. The efficacy of artemether-lumefantrine remains also high in Bangladesh and north-east India.

### **Thailand**

Previously, Thailand used a regimen of 2-day artesunate-mefloquine as first-line treatment. Despite the change to a 3-day regimen in 2009, treatment failures with artesunate-mefloquine increased in Kanchanaburi, Ranong, Tak and Ubonratchathani provinces, reaching a treatment failure rate of  $\geq 10\%$ . The high number of treatment failures observed in Thailand following treatment with artesunate-mefloquine could be attributed to the presence of mefloquine resistance (which has been confirmed countrywide) in addition to artemisinin partial resistance. Mefloquine drug pressure has been considerable over the past few decades, with Thailand using different regimens of mefloquine (15 to 25 mg/kg) as monotherapy or in combination with artesunate. The efficacy of artemether-lumefantrine was evaluated in two provinces in 2012, with treatment failure rates between 6% and 10%. In 2015, dihydroartemisinin-piperaquine was selected as the first-line treatment, and its efficacy is currently being evaluated as part of a system of integrated drug efficacy surveillance.

### **Viet Nam**

Delayed parasite clearance after treatment with dihydroartemisinin-piperaquine was first detected in Viet Nam in the Bu Dang district of Binh Phuoc province in 2009. Routine monitoring with dihydroartemisinin-piperaquine also revealed other foci of delayed parasite clearance in Gia Lai province (2010), Dak Nong province (2011), Quang Nam province (2012), Khanh Hoa province (2014) and Ninh Thuan province (2015). TESs with dihydroartemisinin-piperaquine conducted from 2010 to 2014 found a treatment efficacy of  $>95\%$ , despite a day-3 positivity rate of up to 36%. However, a study in 2015 in Binh Phuoc province reported a high treatment failure rate ( $>10\%$ ) after treatment with dihydroartemisinin-piperaquine. Investigations have confirmed the emergence of piperaquine resistance. In 2016, high treatment failure rates with dihydroartemisinin-piperaquine were reported in Dak Nong province, but resistance to dihydroartemisinin-piperaquine is likely also present in other provinces (Dak Lak and Gia Lai).

## **Africa**

The efficacy of ACTs is being monitored in most malaria-endemic countries. There have been some reports of delayed parasite clearance during routine TESs of ACTs conducted in Africa. However, these reports have not been consistent over time. Artemether-lumefantrine and artesunate-amodiaquine are the first-line treatment policies used in most African countries, with some countries adding



dihydroartemisinin-piperaquine. Between 2010 and 2016, the overall average efficacy rates of dihydroartemisinin-piperaquine, artesunate-amodiaquine and artemether-lumefantrine were 98.7%, 98.3% and 97.9%, respectively.

The presence of multicopy *Pfplasmepsin 2-3* in several African countries (Comoros, Mali, Mozambique, Uganda) is a potential concern, although this mutation has not yet been validated as a molecular marker for piperaquine resistance in African strains.

## South America

A limited number of studies have been conducted in South America, but the efficacy of the ACTs tested remains high. Chloroquine remains the first-line treatment in Mesoamerica and in Hispaniola. In Guatemala, Haiti, Honduras and Nicaragua, molecular marker studies of *Pfcrf*, the marker for chloroquine resistance, have been conducted in lieu of TESs. Between 2010 and 2015, more than 1000 samples were analysed and the mutation was rarely observed. Two TESs conducted in Haiti reported treatment failures; however, molecular analyses were not done to exclude reinfections and no *Pfcrf* mutants were detected in the failing cases.

## Middle East and India

High treatment failure rates with artesunate-sulfadoxine-pyrimethamine have been observed in Somalia and Sudan. This has prompted a change in treatment policy, with both countries adopting artemether-lumefantrine as first-line treatment. These results have been further supported by investigations into the presence of *Pfdhps* and *Pfdhfr* quadruple and quintuple mutations. In Afghanistan, Iran (Islamic Republic of), Pakistan and Yemen, treatment failure rates with artesunate-sulfadoxine-pyrimethamine were found to be less than 10%.

In India, three studies conducted in 2012 detected treatment failure rates >10% with artesunate-sulfadoxine-pyrimethamine in the absence of artemisinin partial resistance. This led to a change in treatment policy to artemether-lumefantrine in the north-eastern part of the country.

## CONCLUSION

Despite the delayed response to artemisinin in some areas of the GMS, ACTs remain the most effective treatment for uncomplicated falciparum malaria. Most patients with delayed parasite clearance are cured, as long as the partner drug remains effective. Routine monitoring must continue in order to ensure that the recommended ACTs are effective, that timely changes to national treatment policies can be implemented, and that artemisinin resistance can be detected early. Assessment of K13 propeller region mutants will greatly facilitate the tracking of artemisinin partial resistance as it emerges. In the context of multidrug resistance, including artemisinin partial resistance and partner drug resistance in the GMS, elimination of falciparum malaria has become a high priority. The role played by artemisinin resistance in the development or selection of partner drug resistance needs to be further evaluated.

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Please also visit the following WHO website for additional information and data:  
[http://www.who.int/malaria/areas/drug\\_resistance/en/](http://www.who.int/malaria/areas/drug_resistance/en/)

**Notes**

1. Artemisinin refers to artemisinin and its derivatives.
2. For further information refer the report of the WHO Evidence Review Group Report on K13 molecular marker of artemisinin resistance: [http://www.who.int/malaria/areas/drug\\_resistance/ERG-K13-molecular-marker-minutes-2014.pdf](http://www.who.int/malaria/areas/drug_resistance/ERG-K13-molecular-marker-minutes-2014.pdf)

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