



Artemisinin and artemisinin-based combination therapy resistance

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STATUS REPORT

KEY MESSAGES

1. Artemisinin¹ resistance is defined as delayed parasite clearance following treatment with an artesunate monotherapy² or with an artemisinin-based combination therapy (ACT). This represents partial/relative 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 resistance to artemisinin and the partner drug. Outside the GMS, treatment failure with ACTs (artemether-lumefantrine, artesunate-amodiaquine and artesunate-sulfadoxine-pyrimethamine) has occurred in the absence of artemisinin resistance mainly due to partner drug resistance.
3. A molecular marker for artemisinin resistance (*PfKelch13*) has been identified, and data collection on the geographical distribution of this marker is helping to improve the global surveillance of artemisinin resistance.
4. There is no evidence that higher levels of artemisinin resistance (full resistance) have emerged.
5. The emergence of multidrug resistance, including artemisinin and partner drug resistance causing ACT failure, and the independent emergence of artemisinin resistance in multiple locations in the GMS have led WHO to recommend the elimination of malaria in this region.

BACKGROUND ON ARTEMISININ RESISTANCE

Definition of artemisinin resistance

Clinical artemisinin resistance¹ is defined as delayed parasite clearance; it represents a partial/relative resistance that has thus far only affected ring-stage parasites. Delayed parasite clearance following treatment with an ACT is of paramount concern to WHO. Nevertheless, the majority of patients who have delayed parasite clearance following treatment with an ACT are still able to clear their infections, as long as the partner drug remains effective.

The identification of the *PfKelch13* (K13) mutations has allowed for a more refined definition of artemisinin resistance that includes information on the genotype. However, we have yet to fully understand which specific mutations within the K13 domain are most associated with artemisinin resistance. The current definition of artemisinin resistance (Box 1) is subject to change based on new evidence.

There is no evidence that higher levels of artemisinin resistance (full resistance) have emerged.

BOX 1

DEFINITION OF ARTEMISININ RESISTANCE

The presence of artemisinin resistance is generally first evaluated during therapeutic efficacy studies (TESs) in which patients receive treatment with an ACT. It can also be evaluated in special clinical studies designed to evaluate artemisinin resistance; for such studies, patients receive artesunate monotherapy alone or before receiving a partner drug. The following definitions apply to both study types:

Suspected endemic artemisinin resistance is defined as:

- $\geq 10\%$ of patients with a half-life of the parasite clearance slope ≥ 5 hours after treatment with ACT or artesunate monotherapy; or
- $\geq 5\%$ of patients carrying K13 resistance-confirmed mutations (listed in Table 1); or
- $\geq 10\%$ of patients with persistent parasitaemia by microscopy at 72 hours (± 2 hours; i.e., day 3).

Confirmed endemic artemisinin resistance is defined as:

- $\geq 5\%$ of patients carrying K13 resistance-confirmed mutations, all of whom have been found to have either persistent parasitaemia by microscopy on day 3 or a half-life of the parasite clearance slope ≥ 5 hours after treatment.

Evaluations of artemisinin resistance take into consideration several confounding factors, including the effect of partner drugs, immunity, insufficient levels of drug in the blood, and non-validated K13 mutations.

Possible consequences of artemisinin resistance

Artemisinin is used in combination with other medicines to quickly reduce the parasite biomass. The consequences of partial/relative resistance could include: 1) the development of total artemisinin resistance; 2) failure to rapidly clear parasites, which could compromise the use of artemisinin for the treatment of severe malaria; and 3) slow parasite clearance in patients treated with an ACT, which could cause more parasites to be exposed to the partner medicine alone once the artemisinin component has been rapidly eliminated following the 3-day treatment course.

Delayed clearance could increase the risk of de novo resistance to the partner drug, particularly in patients with high parasitaemia at admission, and/or facilitate the selection of parasites that are resistant to the partner drug. Treatment failures are likely to increase with resistance to partner drugs. Nevertheless, further research is needed to evaluate the exact role played by artemisinin resistance in the de novo emergence and/or selection of parasites that are less sensitive to partner drugs.

Prevalence of K13 mutations

To date, more than 200 non-synonymous mutations in the K13 gene have been reported. Distinct alleles with K13 mutations originating from multiple independent emergence events have been observed in South-East Asia. The KARMA project has reported frequent C580Y, R539T, Y493H and I543T mutations in the eastern GMS (Cambodia, Lao PDR and Viet Nam) and frequent F446L, N458Y, P574L and R561H mutations in the western GMS (China, Myanmar and Thailand). The P553L allele has been found in the two areas. There is evidence that selective sweeps have occurred throughout the GMS. Currently, the K13 C580Y mutation can be found in several genetic backgrounds (haplotypes) throughout the GMS. The prevalence of one specific K13 C580Y haplotype is increasing, replacing other haplotypes in an area that includes sites in western Cambodia, north-eastern Thailand and southern Lao PDR. This indicates a selective sweep in this part of the GMS. However, the frequencies of different K13 C580Y haplotypes vary by region, and no single haplotype is dominant throughout the GMS.

In Africa, non-synonymous K13 mutations are still rare and highly diverse. A very low prevalence of non-synonymous K13 mutations has been reported in Angola, Burkina Faso, Cameroon, Central African Republic, Comoros, Congo, Côte d'Ivoire, Democratic Republic of the Congo, Equatorial Guinea, Ethiopia, Gabon, Gambia, Kenya, Liberia, Madagascar, Malawi, Mali, Mozambique, Niger, Nigeria, Rwanda, Senegal, Sierra Leone, Togo, Uganda and Zambia.

Validation of a K13 mutation as a resistance marker

Not all non-synonymous propeller-region K13 mutants reported indicate the emergence of artemisinin resistance; rather, such mutants can represent 'passer-by' genotypes if there is no evidence for the selection of the mutant K13 genotype. In addition, different K13 mutations have varying effects on the clearance phenotype. The validation of the K13 mutant as a resistance marker requires it to be correlated with slow clearance in clinical studies, reduced drug sensitivity in ex-vivo assays or in vitro assays (e.g., ring-stage assay – RSA_{0-3h}), or reduced in vitro sensitivity resulting from the insertion of the K13 mutant in transfection studies (see Box 2).

BOX 2

DEFINITION OF CANDIDATE AND VALIDATED K13 RESISTANCE MUTATIONS ASSOCIATED WITH DELAYED CLEARANCE

The impact of different K13 mutations on the clearance phenotype varies. A K13 mutation is defined as a candidate or validated K13 resistance mutation through the confirmation of:

- a statistically significant association ($P < 0.05$) between a K13 mutation and either a half-life of the parasite clearance slope of ≥ 5 hours or positive parasitaemia at 72 hours (± 2 hours) via a chi-squared test or appropriate multivariable regression model on a sample of at least 20 clinical cases; or
- $> 1\%$ survival using the RSA_{0-3h} (or > 2 standard deviations above the mean value for K13 wild-type parasites from the same area) in at least five individual isolates with a given mutation; or a statistically significant difference ($P < 0.05$) in the RSA_{0-3h} assay between culture-adapted recombinant isogenic parasite lines produced using transfection and gene-editing techniques, which express a variant allele of K13 as compared to the wild-type allele.

A K13 mutation is considered validated when both of these requirements have been met, and a candidate when only one of these requirements has been met. However, at this time, the RSA_{0-3h} and the thresholds for in vivo tests have only been validated for South-East Asian parasites and patients.

The current list of candidate (correlated with delayed parasite clearance) and validated (by in vivo and in vitro data) K13 propeller mutations can be found in Table 1 below (this list is updated regularly):

TABLE 1
Candidate and validated K13 resistance mutations*

K13 MUTATION	CLASSIFICATION
<i>E252Q</i>	<i>Not associated</i>
P441L	Candidate
F446I	Candidate
G449A	Candidate
N458Y	Validated
Y493H	Validated
G538V	Candidate
R539T	Validated
I543T	Validated
P553L	Candidate
R561H	Candidate
V568G	Candidate
P574L	Candidate
<i>A578S</i>	<i>Not associated</i>
C580Y	Validated
A675V	Candidate

* Other less frequent variants have been associated with in vivo or in vitro tests, or both: M476I; C469Y; C469F; M476I; K479I; A481V; R515K; S522C; P527L; N537I; N537D; G538V; R575K; M579I; D584V; P667T; F673I; H719N.

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.

RESPONDING TO DECLINES IN DRUG EFFICACY

Monitoring the therapeutic efficacy of ACTs

Therapeutic efficacy studies (TESs) assess clinical and parasitological patient outcomes, providing the main reference for national malaria control programmes (NMCPs) in determining their national malaria treatment policy. To ensure that the treatments recommended in the national treatment policies 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 in order to facilitate early detection of additional resistance foci.

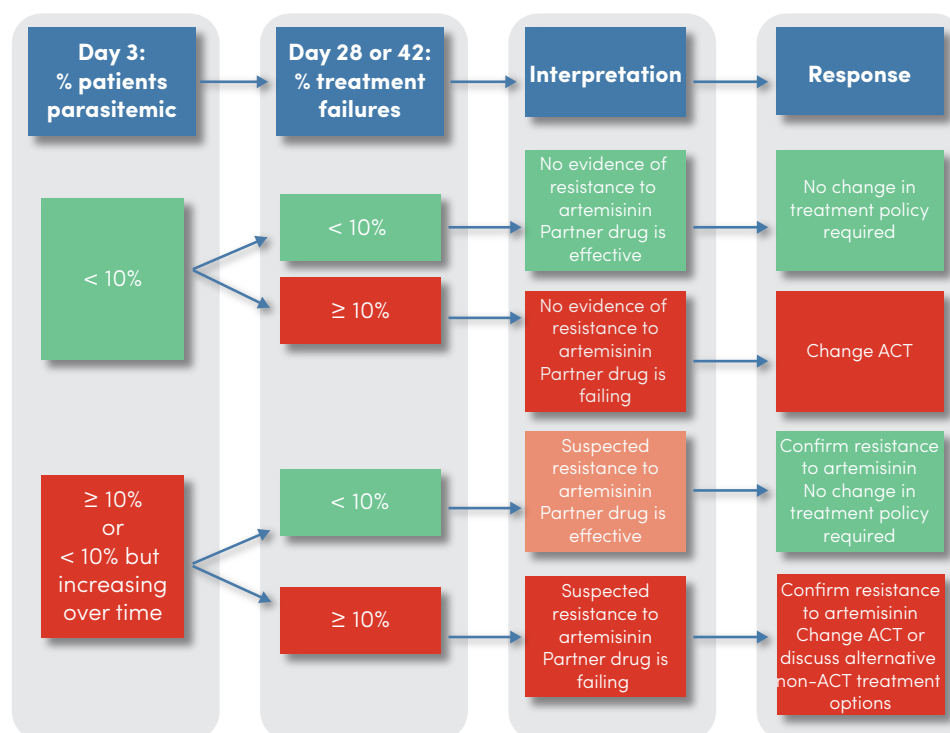
Changing the treatment policy for *P. falciparum*

Nearly all malaria-endemic countries recommend artemisinin-based combination therapies (ACTs) for the treatment of uncomplicated *P. falciparum*. TES results for ACTs 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 resistance in *P. falciparum*;
- the **proportion of treatment failures by day 28 or 42** (days of follow-up is determined according to the half-life of the ACT partner drug).

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

FIGURE 1
Decision-making process based on TES results



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 resistance in the GMS, containment efforts were initiated to stop the spread of resistant parasites through a combination of 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 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 (Figure 2), threatening the progress achieved in this region, where the incidence of malaria has been greatly reduced over the last 10 years. Since elimination in the GMS is considered technically feasible at a reasonable cost, in September 2014, the Malaria Policy Advisory Committee of WHO recommended the adoption of the goal to eliminate *P. falciparum* in the GMS by 2030.

During the World Health Assembly in May 2015, WHO launched the *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 for the period 2018–2020 to support the countries' progress towards the elimination of malaria.

COUNTRY UPDATES ON ACT EFFICACY

South-East Asia

Cambodia

Background

- Artemisinin resistance was first identified in clinical studies in 2006; however, the retrospective analysis of molecular markers has indicated that artemisinin resistance likely emerged prior to 2001 and the widespread deployment of ACTs in Cambodia;
- 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;
- After the implementation of this new treatment policy, an increase in treatment failures was quickly identified through TESs using dihydroartemisinin-piperaquine in Pailin. Between 2008 and 2015, similar trends were observed in nine provinces, mainly in the western and northern parts of the country. The high treatment failure rates observed with dihydroartemisinin-piperaquine are related to the presence of piperaquine resistance (which has been present in Cambodia since 2002) and has spread geographically from western to north-eastern Cambodia;
- A consensus meeting on the national treatment policy for *P. falciparum* was held in January 2014. Artesunate-mefloquine was reintroduced as the first-line treatment, since the proportion of falciparum strains with multiple *Pfmdr1* copy numbers (which confer mefloquine resistance) is currently minimal in the area. Quinine plus doxycycline over 7 days has been adopted as rescue therapy.

Update

- Studies with artesunate-mefloquine conducted between 2014 and 2017 have reported 100% efficacy, even though around 95% of parasites have been found to carry the K13 C580Y mutation;
- In 2017, artesunate-amodiaquine studies reported significant treatment failure rates, which indicates that five ACTs are failing in Cambodia.

Lao PDR

Background

- In 2013, a trial conducted in Champasack province found that 22.2% of the patients treated with artemether-lumefantrine were still parasitaemic on day 3 after treatment, with a 10% treatment failure rate; by contrast, in Saravanh province, the positivity rate on day 3 was 2%, with an overall treatment failure rate of 2.4%;
- The emergence of artemisinin resistance in southern Lao PDR has been supported by the presence of K13 mutants (mainly C580Y and R539T) in the circulating parasite populations, as identified in 2013.

Update

- The therapeutic efficacy of artemether–lumefantrine has begun to be affected; in 2015, TEs conducted in Atteupeu, Sekong and Champasack provinces showed day–3 positivity rates that ranged between 9.7% and 20%, and treatment failure rates that ranged between 0% and 14%. In 2016, the monitoring of dihydroartemisinin–piperaquine began to ensure that data would be available to support a potential alternative ACT for use in the country.

Myanmar

Background

- Artemisinin resistance likely emerged at the border between Thailand and Myanmar in 2001, but it 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).

Update

- Studies evaluating the presence of K13 mutants have shown that the predominant K13 mutant found in Myanmar does not appear to have spread from Cambodia but likely arose independently;
- A K13 F446I propeller polymorphism has been found to be significantly associated with delayed parasite clearance. Surveys indicate that there is a high prevalence of the K13 F446I mutation along the China–Myanmar and India–Myanmar borders. This mutant appears to be associated with an intermediate rate of delayed clearance, and additional in vitro research is ongoing to validate its role in artemisinin resistance;
- The three first–line ACTs used in the country are still effective with high cure rates.
- ACT efficacy remains high on both sides of the border between India and Myanmar.

Thailand

Background

- Previously, Thailand used a regimen of 2–day artesunate–mefloquine as its first–line treatment. Despite the change to a 3–day regimen in 2009, treatment failures with artesunate–mefloquine have increased in Kanchanaburi, Ranong, Tak and Ubonratchathani, reaching a treatment failure rate $\geq 10\%$;
- The high numbers of treatment failure observed in Thailand following treatment with artesunate–mefloquine could be explained by the presence of mefloquine resistance (which has been confirmed countrywide) in addition to artemisinin resistance. Mefloquine drug pressure has been considerable over the past few decades, as Thailand has used 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, revealing treatment failure rates close to or greater than 10%.

Update

- In 2014–2015 in Kanchanaburi province, the efficacy of dihydroartemisinin-piperaquine was 94%;
- During a consensus meeting held in 2015, dihydroartemisinin-piperaquine was chosen as the first-line treatment, and its efficacy is continually being monitored.

Viet Nam

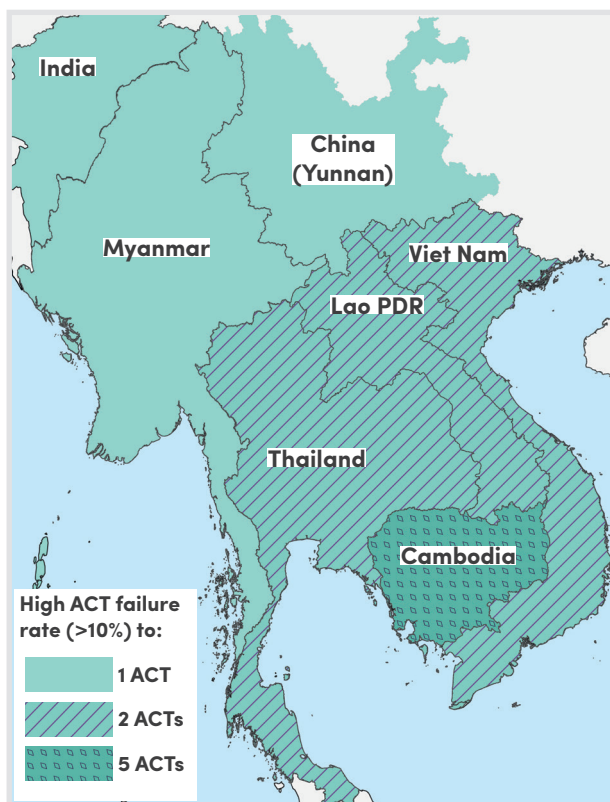
Background

- Delayed parasite clearance was first detected following treatment with dihydroartemisinin-piperaquine in the Bu Dang district of Binh Phuoc province in 2009;
- Routine monitoring with dihydroartemisinin-piperaquine also detected 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 conducted from 2010 to 2014 using dihydroartemisinin-piperaquine have confirmed a treatment efficacy of > 95%, despite a day-3 positivity rate of up to 36%.

Update

- A study in 2015 in Binh Phuoc province reported a high treatment failure rate (> 10%) following treatment with dihydroartemisinin-piperaquine. Investigation has confirmed the emergence of piperaquine resistance.

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 (AS+SP) and dihydroartemisinin-piperaquine. A sixth ACT, artesunate-pyronaridine, was given a positive scientific opinion by the European Medicines Agency (EMA) under article 58 and is being considered for recommendation by WHO. By default, AS+SP is considered to have a high failure rate in the region because of high treatment failure rates with sulfadoxine-pyrimethamine and/or because quadruple and quintuple *Pfdhfr* and *Pfdhps* mutations (which are usually fixed) have been reported in the region.

Africa

- The efficacy of ACTs is being monitored in most malaria-endemic countries. There have been some reports of delayed parasite clearance during routine TEs of ACTs conducted in Africa. However, these reports have not been consistent over time;
- The most frequent allele observed in Africa is A578S. This allele has not been associated with clinical or in vitro resistance to artemisinin;
- A high number of mutations, including some associated with delayed clearance in the GMS (in particular K13 C580Y), have been reported in Africa. However, many of these mutations reported in Africa have not expanded in the African parasite populations;
- A recent case report (3) warned of the emergence of artemisinin resistance in Equatorial Guinea, yet further investigations are needed before the K13 mutation found (M579I) can be confirmed as an artemisinin resistance marker (see Box 2); due to many confounding factors, a single case with incomplete data is not sufficient to confirm the presence of resistance.

South America

Suriname

- Routine surveillance of ACT efficacy between 2005–2006 and 2011 among gold miners showed an increase in the day-3 positivity rate (from 2% to more than 20%), with a high cure rate at day 28. There were however concerns regarding the quality of the microscopy conducted. In 2013–2014, a study using artesunate and mefloquine did not confirm the high day-3 positivity rate, and the sequencing of the K13 strains collected during this study revealed only wild-type K13.

Guyana

- A retrospective analysis of blood samples collected in 2010 for a *Pfhrp2* surveillance study detected K13 C580Y. All five K13 C580Y mutant samples detected had nearly identical haplotypes, suggesting a common origin distinct from the South-East Asian K13 C580Y haplotype. A survey collecting samples for K13 sequencing is currently underway in the region where five of the earlier cases originated;
- A clinical study evaluating 7-day artesunate for uncomplicated falciparum malaria began in 2014. The efficacy of artesunate was found to be 100% at day 28, whereas only 2% of the patients had persistent parasitaemia on day 3 after treatment. The 47 strains collected all showed wild-type K13.

French Guiana

- Between 2009 and 2013, the day-3 positivity rate among patients treated in Cayenne hospital with artemether-lumefantrine was 7.5%; however, the treatment was not systematically supervised. To date, no K13 C580Y mutant has been reported from French Guiana.

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 resistance as it emerges. In the context of multidrug resistance including ACT 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/

Notes

1. Artemisinin refers to artemisinin and its derivatives.
2. Although WHO does not recommend artesunate monotherapy, it can be used in clinical trials.

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