Status report on artemisinin and ACT resistance

September 2015

Key messages

1. Artemisinin resistance[1] is defined as delayed parasite clearance following treatment with an artesunate monotherapy, or after treatment with an artemisinin-based combination therapy (ACT). Such resistance represents partial resistance.

2. Delayed parasite clearance will not necessarily lead to treatment failure. In the Greater Mekong subregion (GMS), a high treatment failure rate following treatment with an ACT has only been observed where there is resistance to the partner drug, regardless of the presence of artemisinin resistance. However, artemisinin resistance could facilitate the selection of partner drug resistance.

3. A molecular marker for artemisinin resistance has been identified and will help to improve the global surveillance of artemisinin resistance.

4. Emergence of multidrug resistance, including ACT resistance, and independent emergence of artemisinin resistance in the GMS have led to the recommendation of elimination of malaria in this region.

Background

Artemisinin resistance

Artemisinin resistance is defined as delayed parasite clearance; this represents a partial resistance that so far affects only ring-stage parasites. Most patients who have delayed parasite clearance following treatment with an artemisinin-based combination therapy (ACT) clear their infections. However, this is not the case in Cambodia and Thailand, where there is concomitant resistance to the partner drugs such as mefloquine and piperaquine.

It is not clear whether artemisinin resistance has precipitated the emergence of piperaquine resistance, or whether it has helped to further select parasites that are already piperaquine resistant. Resistance to piperaquine may have emerged independently from resistance to artemisinin, because of the long half-life of piperaquine and its previous use as a monotherapy (similar to the use of mefloquine). Further research is needed to evaluate the exact role of artemisinin resistance in the development or selection of drug resistance to partner drugs.

Molecular marker of artemisinin resistance

A molecular marker of artemisinin resistance was recently identified. Mutations in the Kelch 13 (K13) propeller region are associated with delayed parasite clearance both in vitro and in vivo. The identification of **the** K13 marker for artemisinin resistance has allowed for a more refined definition of resistance that includes information on the genotype. However, as the list of mutations associated with artemisinin resistance is still evolving, so the definition of artemisinin resistance will continue to evolve. The current definition of artemisinin resistance is divided into:

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1. Artemisinin refers to artemisinin and its derivatives.

- *suspected artemisinin resistance* – defined as a high prevalence of the delayed parasite clearance phenotype, or high prevalence of K13 mutants; and
- *confirmed artemisinin resistance* – defined as a combination of delayed parasite clearance and K13 resistance-associated mutations in a single patient.

Confounding factors in these definitions include the effect of partner drugs, immunity, insufficient levels of drug in the blood and non-validated K13 mutations.

A total of 186 K13 alleles, including 108 non-synonymous mutations,[2][3] have been reported so far. In South-East Asia, distinct alleles originating from multiple independent events of emergence have been observed. In the eastern Greater Mekong subregion (GMS) – comprising Cambodia, Lao People's Democratic Republic (PDR) and Viet Nam – the mutations C580Y, R539T, Y493H and I543T are frequent. In the western GMS – comprising China, Myanmar and Thailand – mutations F446L, N458Y, P574L and R561H are common. In Africa, non-synonymous mutations are rare but highly diverse. Non-synonymous K13 mutations have been reported in Cameroon, Central African Republic, Chad, Comoros, Democratic Republic of the Congo, Gabon, Gambia, Kenya, Madagascar, Malawi, Mali, Rwanda, Togo, Uganda and Zambia. The most frequent allele observed in Africa is A578S.

Not all non-synonymous propeller-region K13 mutants reported indicate emerging artemisinin resistance. Such mutants can represent "passer-by" genotypes; that is, they do not lead to selection of the mutant K13 genotype. In addition, the position of the mutation affects the clearance phenotype. Validation of a K13 mutation as a resistance marker will require correlation with slow clearance in clinical studies, reduced drug sensitivity in ex vivo or in vitro assays (e.g. the ring-stage assay – RSA_{0-3h}), or reduced in vitro sensitivity in transfection studies involving insertion of the mutant K13. Table 1 provides a list (that will have to be updated regularly) of associated K13 propeller mutations (i.e. those correlated with delayed parasite clearance) and confirmed K13 propeller mutations (i.e. those confirmed by in vivo and in vitro data).

Table 1. Associated and validated K13 resistance mutations

K13 mutation	Classification
441L	Associated
446I	Associated
449A	Associated
458Y	Associated
493H	Confirmed
539T	Confirmed
543T	Confirmed
553L	Associated
561H	Associated
568G	Associated
574L	Associated
580Y	Confirmed
675V	Associated

Monitoring therapeutic efficacy of ACTs

Routine monitoring of the therapeutic efficacy of ACTs is essential for making timely changes to treatment policy; it can also help to detect early changes in *Plasmodium falciparum* susceptibility to antimalarial drugs. WHO currently recommends monitoring the efficacy of first-line and second-line ACTs every 2 years in all falciparum-endemic countries. The results of therapeutic efficacy studies (TESs) make it possible to determine the:

- *proportion of patients who are parasitemic on day 3,* which is currently the indicator of choice for routine monitoring to identify suspected artemisinin resistance in *P. falciparum*; and

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2. A non-synonymous mutation is one that changes the gene expressed and the phenotype of the individual.
3. Preliminary data of the KARMA Project initiated by Institut Pasteur in collaboration with WHO.

- *proportion of treatment failure* by 28-day or 42-day follow-up (depending on the partner drug half-life in the specific ACT); a treatment failure rate exceeding 10% should prompt a change in the national antimalarial treatment policy.

The flowchart in Fig. 1 outlines the recommended steps in the decision-making process for the interpretation of and response to TES findings.

Figure 1. Decision-making process based on TES results
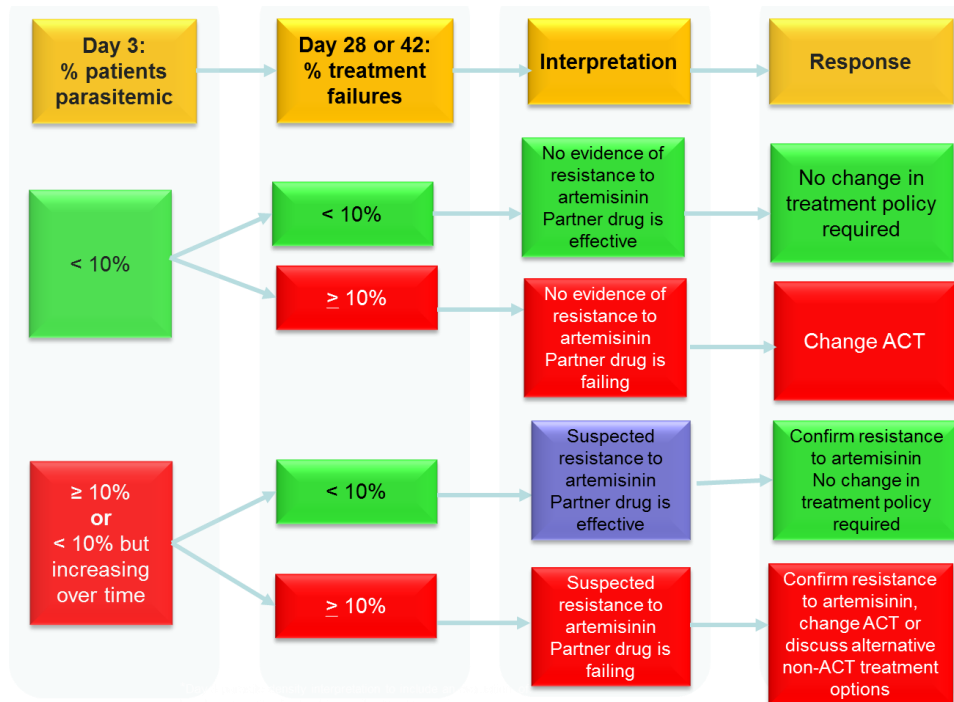
If artemisinin resistance is suspected because of slow clearance in a clinical trial or TES, K13 marker analysis (e.g. from filter-paper blood spots) should be prioritized. If resistance is suspected based on a survey with molecular data only, it should be confirmed by studies that combine information on the clinical phenotype (delayed parasite clearance) and the K13 genotype from the same parasite strain. If necessary, the K13 mutant should be validated as a resistance marker using an in vitro assay such as the RSA_{0-3h}.

Possible implications of delayed parasite clearance

Delayed parasite clearance after treatment with an ACT is of great concern. Failure to rapidly clear parasites could compromise the use of artemisinin for the treatment of severe malaria. Also, slow parasite clearance causes more parasites to be exposed to the partner medicine alone, increasing the risk of selection of partner drug resistance, which in turn increases the risk of treatment failure. Currently, most patients with a delayed parasite clearance response are still cured by ACTs, provided that the partner drug remains effective. Finally partial resistance could include development of total artemisinin resistance.

Response to artemisinin resistance and eliminating malaria in the GMS

Emergency response to artemisinin resistance in the GMS

In April 2013, WHO launched the *Emergency response to artemisinin resistance (ERAR) in the GMS* (*1*).The framework urges partners to work in a coordinated manner to provide malaria interventions to all at-risk groups; to achieve tighter coordination and management of field operations; to obtain better information for containment of artemisinin resistance; and to strengthen regional oversight and support.

WHO has received support from the Australian Department of Foreign Affairs and Trade and the Bill & Melinda Gates Foundation to strengthen the coordination of and technical support for activities to contain artemisinin resistance in the GSM. The project is implemented by the WHO Global Malaria Programme, the WHO Regional Office for South-East Asia, the WHO Regional Office for the Western Pacific and WHO country offices. A regional hub has been established in Phnom Penh, Cambodia, to support and help with coordination of activities.

In line with the call to action and recommendations contained in the ERAR, the Global Fund to Fight AIDS, Tuberculosis and Malaria has allocated US$ 100 million to a regional artemisinin initiative, funding activities to contain and eliminate artemisinin resistance in Cambodia, Lao PDR, Myanmar, Thailand and Viet Nam. The regional artemisinin initiative includes a regional component to support cross-border activities.

Malaria elimination in the GMS

The incidence of malaria has been greatly reduced over the past 10–20 years. However, there is concern that falciparum malaria in the GMS is becoming increasingly resistant to antimalarial medicines; at the border between Cambodia and Thailand, falciparum malaria could become untreatable within a few years. In addition, molecular studies have confirmed that artemsinin resistance has emerged independently in many areas of the GMS. Against this background, WHO's Malaria Policy Advisory Committee recommended in September 2014 the adoption of the goal of elimination of *P. falciparum* in the GMS by 2030. Subsequently, at the World Health Assembly in May 2015, WHO launched a *Strategy for malaria elimination in the GMS (2015–2030)* (*2*), which was endorsed by all the GMS countries.

Country updates on ACT efficacy

The information given here is taken from the *Global report on antimalarial efficacy and drug resistance: 2000–2010* (*3*). In addition WHO regularly provides updates on the global status of antimalarial drug efficacy of both *P. falciparum* and *P. vivax*, through reports and maps which are regularly updated on the WHO website (http://www.who.int/malaria/areas/drug_resistance/maps/en/).

South-East Asia

Cambodia

Background

- Artemisinin resistance was first identified in clinical studies in 2006; however, retrospective analysis of molecular markers indicates that artemisinin resistance probably emerged in 2001, before the widespread deployment of ACTs in Cambodia.

- Due to high failure rates with artesunate-mefloquine (ASMQ), the first-line treatment for the treatment of uncomplicated falciparum malaria, was changed from co-blistered ASMQ to fixed-dose dihydroartemisinin-piperaquine (DHA-PPQ) 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 in TESs using DHA-PPQ in Pailin. Between 2008 and 2014, similar trends were observed in seven provinces, mainly in the western and northern part of the country. The high treatment failure rates observed with DHA-PPQ are related to the presence of piperaquine resistance, which is spreading from western to north-eastern Cambodia.

- A consensus meeting held in November 2011 recommended the use of atovaquone-proguanil delivered as directly-observed therapy for Pailin province as a short-term interim solution, with stringent follow-up for monitoring resistance. Mutations conferring resistance to atovaquone were observed less than a year after the implementation of the drug as first-line therapy, which was sufficient reason to change the recommendation.

- A consensus meeting on the national treatment policy for *P. falciparum* was held in January 2014. ASMQ was re-introduced as 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. DHA-PPQ remains the first-line treatment in the rest of the country.

Lao PDR

Update

- In 2013, a trial conducted in Champasack province found that 22.2% of the patients treated with artemether-lumefantrine (AL) were still parasitemic on day 3 after treatment.

- The emergence of artemisinin resistance in southern Lao PDR is supported by the identification in 2013 of the presence of K13 mutants (mainly C580Y and R539T) in the circulating parasite populations.

- The therapeutic efficacy of AL has not been affected, and cure rates have remained high since 2005.

- Containment activities started in 2014, and TESs are now being conducted in Atteupeu, Champasack and Sekong provinces.

Myanmar

Background

- Artemisinin resistance probably emerged at the border between Myanmar and Thailand in 2001, but was not clearly recognized until 2008.

- Since 2009, data show consistently delayed parasite clearance times among a significant proportion of patients treated with ACTs; this trend was observed in all the three first-line ACTs (AL, ASMQ and DHA-PPQ).

- The results showing delayed parasite clearance rates in several parts of the country led to the initiation of the Myanmar Artemisinin Resistance Containment framework, in line with the recommendations described in the *Global Plan for Artemisinin Resistance Containment (GPARC)* (*4*).

- The three first-line ACTs used in the country are still effective, with high cure rates.

Update

- Studies evaluating the presence of K13 mutants have shown that the predominant K13 mutant found in Myanmar is likely to have arisen independently rather than to have spread from Cambodia.

- A new K13 propeller polymorphism (F446I) is potentially associated with delayed parasite clearance. Preliminary results indicate a high prevalence of the K13 F446I mutation along both the China–Myanmar border and the India–Myanmar border. Research is ongoing to validate the role of this new mutant in artemisinin resistance.

- ACT efficacy remains high on both sides of the border between India and Myanmar.

Thailand

Background

- Containment activities on the Thailand side of the border between Cambodia and Thailand began simultaneously with those in Cambodia in 2008.

- Thailand initially used a regimen of 2-day ASMQ as first-line treatment. Despite the change to a 3-day regimen in 2009, treatment failures with ASMQ increased in Kanchanaburi, Ranong, Tak and Ubonratchathani, reaching levels of at least 10%.
- High treatment failure rates observed in Thailand after treatment with ASMQ 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 last decades, since Thailand has been using different regimens of mefloquine (15 to 25 mg/kg) as monotherapy or in combination with artesunate.

Update

- The efficacy of AL was evaluated in two provinces in 2012; the treatment failure rate was close to or exceeded 10%.
- During a consensus meeting held in 2015, DHA-PPQ became the first-line treatment in the country, and its efficacy is currently being evaluated.

Viet Nam

Background

- Delayed parasite clearance was first detected after treatment with DHA-PPQ in the Bu Dang district of Binh Phuoc province in 2009.
- Routine monitoring of treatment with DHA-PPQ also detected other foci of delayed parasite clearance in Gia Lai province (2010), Dak Nong province (2011), Quang Nam province (2012), and Kon Tum and Khanh Hoa provinces (2014).
- In mid-2011, Viet Nam began containment activities following GPARC recommendations with the support of the WHO Western Pacific Regional Office and the WHO country office.

Update

- TES conducted since 2010 using DHA-PPQ reported a treatment efficacy of more than 95%, despite a day-3 positivity rate of up to 36%.

Summary

The status of artemesinin resistance in the GMS is summarized in Table 2.

Table 2. Summary of the status of artemisinin resistance in the GMS

	Artemisinin resistance		Containment activities started	AL		AS-MQ		DHA-PPQ	
	Suspected year of emergence	Detected		D3+	TF	D3+	TF	D3+	TF
Cambodia	2001[a]	2006	2009	♦	♦	♦	o	♦	♦
Lao PDR	2013	2013	2014	♦	o	ND	ND	ND	NS
Myanmar	2001[a]	2008	2011	♦	o	♦	o	♦	o
Thailand	2001[a]	2008	2009	♦	♦	♦	♦	♦	
Viet Nam	2009	2009	2011	ND	ND	ND	ND	♦	o

AL, artemether-lumefantrine; AS-MQ, artesunate-mefloquine; D3, day 3; DHA-PPQ, dihydroartemisinin-piperaquine; PDR, People's Democratic Republic

Orange shading indicates first-line treatment; ♦, observed to be >10%; o, observed to be <10%; ND, undetermined

[a] detected retrospectively using molecular markers or retrospective data

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, but these reports have not been consistent over time.
- To date, the K13 mutations observed have not been associated with slow parasite clearance. Currently, Africa appears to be free of the resistance-associated Asian alleles.
- TESs show that, in general, ACTs remain efficacious.

South America

Suriname

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

Guyana

- The last TES study evaluating AL was conducted from May 2011 to July 2012; a total of 92 patients were enrolled, with 68 completing the day-28 follow-up. A total of 70.8% of day-3 slides were reported to be positive, but after a review of quality control, this result was considered to be flawed. A new clinical study evaluating 7-day artesunate for uncomplicated falciparum malaria was started in 2014. The efficacy of artesunate was 100% at day 28, whereas only 2% of the patients had persistent parasitaemia at day 3 after treatment. The 47 strains collected all showed K13 wild type.
- A retrospective analysis of blood samples collected in 2010 for a histidine rich protein-2 (HRP2) surveillance study, detected the C580Y mutation. All five C580Y mutant samples detected had a nearly identical haplotype, suggesting that they had a common origin that was distinct from the South-East Asian C580Y haplotype. A survey for K13 sequencing is ongoing in the region where five of the earlier cases originated.

French Guyana

- Between 2009 and 2013, the day-3 positivity rate among patients treated in Cayenne Hospital after treatment with AL was 7.5%, but the treatment was not systematically supervised. So far, no K13 mutant strains have been reported from French Guyana.

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 to ensure that the recommended ACTs are effective, that changes in national treatment policies can be implemented in a timely manner, and that artemisinin resistance can be detected early. Assessment of K-13 propeller-region mutants will greatly facilitate the tracking of artemisinin resistance as it emerges. Due to the existence 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 of partner drug resistance needs to be further evaluated.

Further information

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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/index.html

References

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