

Update on artemisinin resistance - September 2011

There is concern over the emergence and possible spread of *Plasmodium falciparum* resistance to artemisinins.¹ In January 2011, the *Global Plan for Artemisinin Resistance Containment* (GPARC) was released to outline the actions required to deal with the threat of artemisinin resistance. This note aims to: reiterate key points from the GPARC, provide background and updates on the current situation of artemisinin resistance in affected countries in the Mekong region, summarize current activities and recommend further action where needed.

Background

Routine monitoring

Routine monitoring of the therapeutic efficacy of artemisinin-based combination therapies (ACTs) is essential for timely changes to treatment policy and can help to detect early changes in *Plasmodium falciparum* sensitivity to artemisinins. WHO currently recommends a change in antimalarial treatment policy when the treatment **failure rate** of a 28- or 42-day follow-up study (depending on the medicine) exceeds 10%. The **proportion of patients who are parasitemic on day 3** is currently the best available indicator used in routine monitoring to measure *P. falciparum* sensitivity to artemisinins. If $\geq 10\%$ of patients treated with an ACT are parasitemic on day 3, the area will be considered Tier I², and, consistent with recommendations in the GPARC, containment activities should begin immediately. Carefully controlled therapeutic efficacy studies using oral artesunate monotherapy should also be initiated to further confirm and investigate the presence of artemisinin resistance in the area. Confirmation of artemisinin resistance should not delay containment activities.

Defining artemisinin resistance

The working definition of artemisinin resistance is based on clinical and parasitological outcomes observed during routine therapeutic efficacy studies of ACTs and clinical trials of artesunate monotherapy:

- an increase in parasite clearance time, as evidenced by $\geq 10\%$ of cases with parasites detectable on day 3 after treatment with an ACT (suspected resistance)³;

or

- treatment failure after treatment with an oral artemisinin-based monotherapy with adequate antimalarial blood concentration, as evidenced by the persistence of parasites for 7 days, or the presence of parasites at day 3 and recrudescence within 28/42 days (confirmed resistance).

¹ Artemisinin refers to artemisinin and its derivatives.

² WHO (2011). Global plan for artemisinin resistance containment.

http://www.who.int/malaria/publications/atoz/artemisinin_resistance_containment_2011.pdf

³ Stepniewska K. et al. (2010). *Journal of Infectious Diseases*. 201(4):570-9.

The definition is likely to be adapted over time, for instance when molecular markers or better in vitro laboratory tests for artemisinin resistance become available. The current definition is also subject to potential confounding factors (i.e. splenectomy, haemoglobin abnormalities and reduced immunity), which can also delay parasite clearance.

The delayed response after a treatment with an ACT is of paramount concern to WHO. The unique ability of artemisinins to clear parasites rapidly is well known; it has been considered to be their 'pharmacodynamic hallmark'. Failure to rapidly clear parasites will compromise their use for the treatment of severe malaria and for treatment of uncomplicated falciparum malaria with ACTs. It causes more parasites to be exposed to the partner medicine alone, increasing the risk of resistance developing to the partner medicine. If resistance develops to the partner medicine, treatment failures are likely to increase. Most patients with delayed response are cured provided that the partner drug remains effective.

*Global Plan for Artemisinin Resistance Containment (GPARC)*⁴

The GPARC was established in response to confirmation of artemisinin-resistance in Cambodia and Thailand, and concerns that resistance could either spread or emerge spontaneously elsewhere. The primary objective of GPARC to protect ACTs as an effective treatment for *P. falciparum* malaria. The GPARC defined three areas of artemisinin resistance:

TIER I - areas for which there is credible evidence of artemisinin resistance, where an immediate, multifaceted response is recommended to contain or eliminate resistant parasites as quickly as possible;

TIER II - areas with significant inflows of mobile and migrant populations from tier I areas or shared borders with tier I areas, with intensified malaria control to reduce transmission and/or limit the risk of emergence or spread of resistant parasites;

TIER III - *P. falciparum* endemic areas which have no evidence of artemisinin resistance and have limited contact with tier I areas, where prevention and preparedness should focus on increasing coverage with parasitological diagnostic testing, quality-assured ACTs and vector control.

Countries should routinely monitor the therapeutic efficacy of their first- and second line-drugs in all the sentinel sites every two years, in order to promptly detect signs of emerging resistance and to keep their policy relevant. In addition to assessment of the 28- or 42-day cure rates, this should also include information on parasite clearance rate, measured as the proportion of patients still parasitemic 72 hours (3 days) after start of treatment. Based on the results countries should classify their region into one of the three tiers as listed above.

⁴ WHO (2011). Global plan for artemisinin resistance containment.

http://www.who.int/malaria/publications/atoz/artemisinin_resistance_containment_2011.pdf

Summary by country

Main source: Global report on antimalarial efficacy and drug resistance: 2000-2010

Cambodia

Background

- Between 2001 and 2007, the proportion of patients parasitemic on day 3 after treatment with either artemether-lumefantrine or artesunate-mefloquine exceeded 10% in western part of Cambodia, including Pailin, Battambang, and Kampot provinces;
- A research study conducted in 2006 in Tasanh (Battambang province) confirmed two cases of treatment failure after 7 days of artesunate treatment with delayed parasite clearance time and adequate plasma concentration of artesunate and dihydroartemisinin;
- These two findings led to additional studies with artesunate monotherapy (7 days) which were conducted in Pailin (Pailin province) and Tasanh (Battambang province) between 2007 and 2008 and which confirmed delayed parasite clearance in more than 40% of the patients and the emergence of artemisinin resistance;
- In response, a containment project was started in 2008 in zone 1 (tier I) including Pailin, Battambang, Pursat and Kampot provinces;
- First-line treatment was changed from co-blistered artesunate-mefloquine to fixed-dose dihydroartemisinin-piperaquine in tier I;
- The efficacy of artesunate-mefloquine, the first-line treatment in eastern Cambodia remained high (> 95%).

Update⁵

- After the implementation of the containment project, the number of falciparum malaria patients has been reduced significantly, but in the presence of continued artemisinin drug pressure, the proportion of patients parasitemic on day 3 after treatment with dihydroartemisinin-piperaquine increased from 26% to 45% between 2008 and 2010;
- An increasing trend of treatment failures with dihydroartemisinin-piperaquine was reported in Pailin during the same period (from 8.1 to 27.6%), although these numbers are based on a small number of treated patients in 2010;
- In addition, increased proportions of treatment failures (10.7%) with the same drug combination were reported in 2010 in Pursat province, south of Pailin province;
- Monitoring of dihydroartemisinin-piperaquine efficacy throughout Cambodia shows that this ACT remains highly effective in the other parts of the country and is also highly effective against vivax malaria nation wide.

⁵ The information included in the Update paragraphs are data that are new and not included in the Global report on antimalarial efficacy and drug resistance: 2000-2010 (WHO, 2010).

<http://www.who.int/malaria/publications/atoz/9789241500470/en/index.html>

Interpretation of the data

- The increase in the proportion of patients parasitemic on day 3, may be a result of the containment efforts: as the number of falciparum malaria cases decreases, the more resistant parasites will have a higher likelihood of survival, resulting in selection of the resistant parasites;
- The high treatment failures observed with dihydroartemisinin-piperaquine in Pailin and Pursat are worrying and could be related to an emergence of piperaquine resistance, a drug which is related to chloroquine.

Way forward

- Because of the very limited alternative treatment options, *P. falciparum* resistance against piperaquine has far reaching consequences and needs urgent confirmation with inclusion of drug levels in vitro sensitivity testing and eventually molecular markers. If resistance to piperaquine is confirmed, this could seriously compromise the containment efforts. Alternative treatment options include:
 - Pyramax (artesunate-pyronaridine), which has been registered by the Korean FDA and has been submitted to the European Medicines Agency (EMA) for an opinion;
 - Quinine-doxycycline for 7 days. Disadvantage of this regimen is poor tolerability resulting in poor compliance and therefore difficult implementation;
 - Atovaquone-proguanil (which is prone to quick development of resistance);
- A consensus meeting is urgently needed to decide on optimal treatment scenarios for western Cambodia;
- Cambodia was successful in the application of its Global Funds for Aids, Tuberculosis and Malaria (GFATM) round 9 focusing on containment of artemisinin resistance. With this grant, activities started under the Bill & Melinda Gates Foundation (BMGF) funded project will be continued.

Laos

Background

- No cases of delayed parasite responses to artemether-lumefantrine (the first line treatment in Laos) were reported in Laos during routine monitoring between 2002-2007 and this ACT remained highly efficacious.

Update

- In 2011, a trial conducted in Savannakhet province confirmed that all patients were cleared of parasites within 48 hours after treatment with artesunate.

Myanmar

Background

- In 2009, preliminary data suggested delayed parasite clearance in Kawthaung Township (Tanintharyi Region in south-eastern Myanmar bordering Thailand) with 8%

- of patients still parasitemic on day 3 following treatment with artesunate-lumefantrine and 18% following treatment with dihydroartemisinin-piperaquine;
- The overall 28-day treatment failure rates from all studies conducted between 2007-2010 were below 10%;

Update

- A 7-day artesunate monotherapy study has been conducted in Kawthaung in 2011 confirming a high rate of patients (27%) still parasitemic at day 3. Only one patient presented with a late treatment failure during the 28-day follow-up. Pharmacokinetics and molecular studies are on-going.
- During routine monitoring conducted in 2010 in sentinel sites, the study in Mon State (south-eastern Myanmar bordering Thailand) showed that 28% of patients still carried parasites at day 3 following treatment with dihydroartemisinin-piperaquine. These data are currently being validated;
- Other studies performed in 2011 in northern and western parts of Myanmar show that <3% of patients remain parasitemic on day 3 and all studies show low treatment failure rates <10% after 28-days of follow-up, including the above mentioned study in Mon State;
- The results showing delayed parasite clearance rates in several parts of the country led to the initiation of a Myanmar Artemisinin Resistance Containment (MARC) plan, based on the action points designed for tier I and tier II areas described in the GPARC. This containment project is planned to start in September 2011, funded by the donor consortium 'Three Diseases Fund'. Funding for the project has been granted till June 2012;
- Myanmar will apply for a GFATM Round 11 grant which could fund the containment project in south-eastern Myanmar.

Interpretation of the data

- Available data consistently show delayed parasite clearance times suggesting emergence of artemisinin resistance in south-eastern Myanmar;
- the three first-line ACTs used in the country (artesunate-mefloquine, artemether-lumefantrine and dihydroartemisinin-piperaquine) are still effective as treatment for uncomplicated falciparum malaria.

Way forward

- Funding for containment is currently only available until June 2012. If the application for GFATM round 11 is successful, there is still the threat of a funding gap of one year from July 2012-June 2013. Additional funding will be needed to bridge this gap.

Thailand

Background

- Until 2008, Thailand used a regimen of 2-day artesunate-mefloquine as first-line treatment. As a consequence, results of routine monitoring of the 2-day first-line ACT used in sentinel sites are difficult to compare with day 3 positivity rate from data compiled in neighbouring countries. Nevertheless it is noticeable that in Trat province bordering Cambodia, the mean parasite clearance time increased from 2 to 3.7 days between 2003-2007;

- Containment activities at the Thailand side of the border between Cambodia and Thailand were started simultaneously with Cambodia in 2008;
- The proportion of patients positive at day 3 in sentinel sites along the border between Thailand and Myanmar ranged between 0-20%, with foci in Ranong, Tak and Kanchanaburi showing proportions >10%. Therefore, the presence of parasites resistant to artemisinin is also highly suspected at the border between Thailand and Myanmar.

Update

- Despite the change to a 3-day regimen, treatment failures with artesunate-mefloquine increased in Tak and Ranong provinces. In Tak, the efficacy after 42-day follow-up decreased slightly from 96.8% in 2008 to 90.4% and 91.2% in 2009 and 2010, respectively. Similarly, the efficacy in Ranong decreased from 96.8% in 2008 to 87.5% and 90.9% in 2009 and 2010, respectively.

Interpretation of data

- Higher treatment failures observed in Thailand with artesunate-mefloquine could be explained by the presence of mefloquine resistance (which has been confirmed countrywide) on top of reduced artesunate susceptibility. Drug pressure with mefloquine 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 .

Way forward

- The first line treatment for Thailand is currently using a loose combination of artesunate and mefloquine. Consensus is urgently needed on optimal treatment scenarios for Thailand. Possibilities include dihydroartemisinin-piperaquine or fixed dose combination artesunate-mefloquine.
- Thailand was successful in the application of its GFATM round 10 focusing on containment of artemisinin resistance countrywide. With this grant, activities started under the BMGF project will be continued at the border between Thailand and Cambodia and will be started at the border between Thailand and Myanmar.

Viet Nam

Background

- In Bu Dang district of Binh Phuoc province, the proportion of patients still parasite positive at day 3 after artesunate monotherapy or dihydroartemisinin-piperaquine was reported to be 15% and 18% in 2009 and 2010 respectively (National Institute of Malaria, Parasitology and Entomology).
- Routine monitoring has not detected any other foci of reduced susceptibility to artemisinins in the rest of the country.

Update

- In 2011, another research team in Phuoc Long district located in the same province of Binh Phuoc reported similar high proportions (22-28%) of patients still parasite positive at day 3 after artesunate monotherapy or dihydroartemisinin-piperaquine.

- More detailed analysis of these studies performed in 2011, including studies on pharmacokinetics and molecular markers, is currently under way to obtain more accurate assessment of the presence of artemisinin resistance.

Way forward

- In mid 2011, Viet Nam begun containment activities based on the GPARC document with the support from WHO Western Pacific Regional Office and country office;
- A limited amount of funding has been provided by WHO HQ (200,000 \$US over 2 years);
- Viet Nam is currently applying for a GFATM round 11 grant which could fund containment activities.

Research needed to refine the definition of artemisinin resistance

- Most research groups find that standard in vitro tests assessing artemisinin sensitivity do not correlate well to measures of parasite clearance in patients, including day 3 positivity rates. A modified test screening the activity of artemisinin on ring stage parasites is under development;
- The measurement of artemisinin concentrations in whole blood or within the parasitized erythrocyte (where the drug action takes place), might be more relevant than the assessment of plasma concentrations with respect to the observed differences in parasite clearance. New methodologies measuring the concentration in whole blood are being validated to allow a better analysis of the clinical results;
- In western Cambodia, it has been shown that prolonged parasite clearance time is to a large extent explained by a heritable trait of the parasite. However, the genes responsible for artemisinin resistance are still unknown. Molecular studies looking at mutations across the whole parasite genome are on-going and have thus far shown that the genetic basis of artemisinin resistance is likely multigenic, linked to clusters of significant SNPs on multiple chromosomes.
- An in vivo parasitological marker less prone to variation than the proportion of cases parasite positive at day 3 is the parasite clearance rate, which is the slope of the log-linear parasite clearance curve and is independent on the initial parasitaemia. An on-line version converting parasite clearance data into a clearance rate or 'parasite half life' is currently developed and provides a uniform method to describe the delayed clearance phenotype and its relation to resistance.

Conclusion

Despite the delayed response to artemisinin in some areas of the Greater Mekong subregion, ACTs remain the most effective treatment for uncomplicated falciparum malaria; most patients with delayed response are cured if the partner drug remains effective. Nevertheless, WHO is concerned with the growing evidence of resistance, as defined by delayed parasite clearance times, in south-eastern Myanmar and western Thailand and in Binh Phuoc province in Viet Nam. It is not known if these new foci represent spread or de novo emergence of artemisinin resistance. In response to the

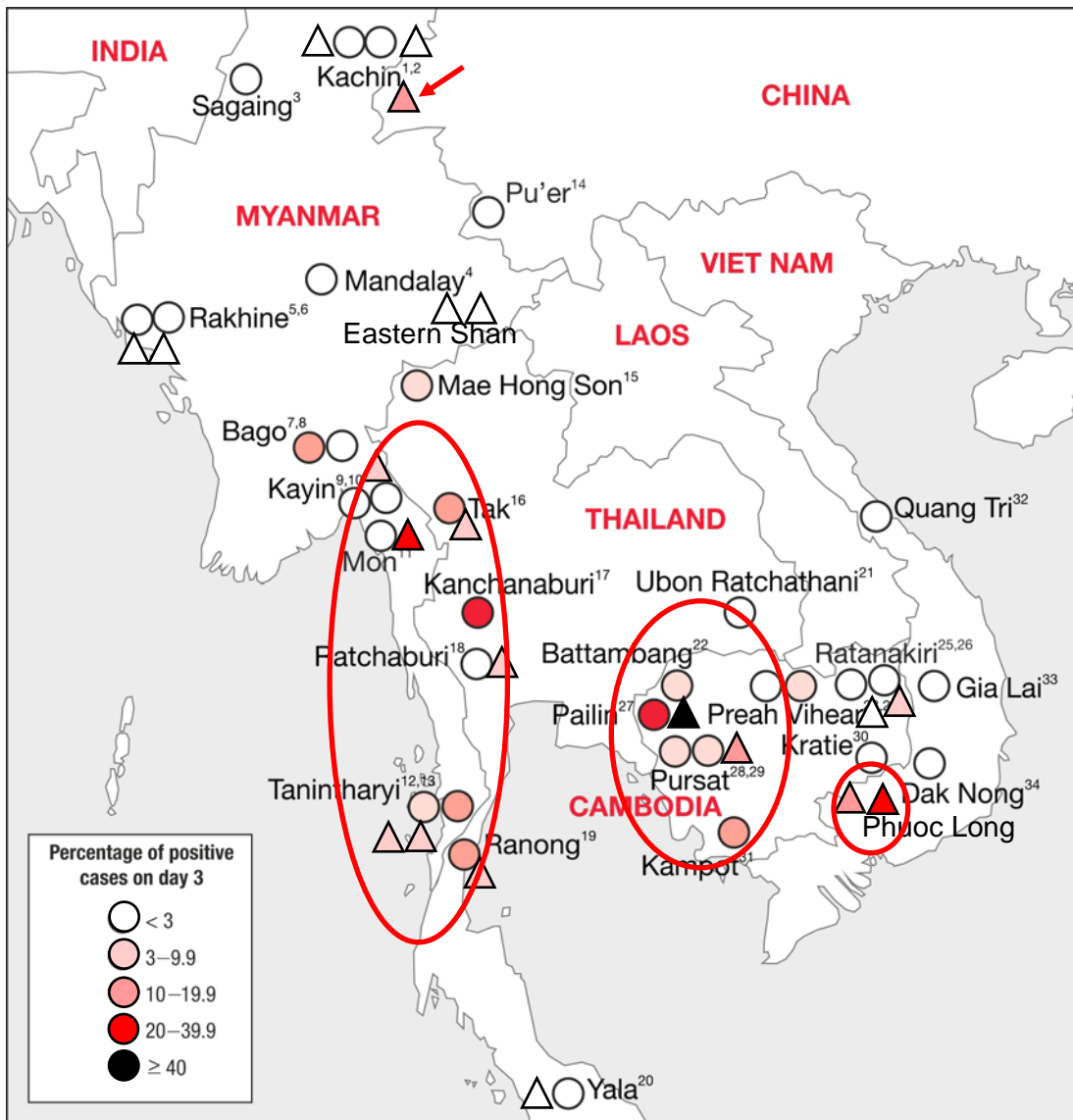
new data, containment projects are planned in western Thailand, south-eastern Myanmar and Viet Nam that will draw lessons learned from the containment project in Cambodia and Thailand, as well as the GPARC. Additional funding will be needed to ensure that the containment projects initiated can be sustained. Furthermore, as artemisinin resistance is prevalent in border areas and migration is known to be a contributing factor in the spread of resistance, there is a need to increase cross-border coordination between national projects and programmes.

Routine monitoring must be continued to ensure that the recommended first line treatments are effective and that timely changes in treatment policies can be made, and to detect the emergence of artemisinin resistance. Many aspects of artemisinin resistance are still not well understood. Consequently, there is an urgent need for further research to refine our knowledge of artemisinin resistance, including the identification of molecular markers and better in vitro sensitivity tests .

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Figure. Percentage of positive cases on day 3 after ACT



Circles represent data before November 2010 and triangles data after November 2010.