WHO-EM/MAL/377/E

Report on the

Sixth intercountry meeting of national malaria control programme managers from HANMAT and PIAM-Net countries

Cairo, Egypt
13–14 August 2014

World Health Organization
Regional Office for the Eastern Mediterranean

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Document WHO-EM/MAL/377/E/11.14

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1. INTRODUCTION

The spread of resistance to antimalarial drugs presents a major challenge to health systems. To meet this challenge, there is need for updated quality information on antimalarial drug treatment efficacy for policy-makers. Monitoring the therapeutic efficacy of antimalarial medicines can generate the information needed for evidence-based malaria treatment policies.

The World Health Organization (WHO) has established two subregional networks for countries endemic with *falciparum* malaria: the Horn of Africa Network for Monitoring Antimalarial Treatment (HANMAT) comprising Djibouti, Saudi Arabia, Somalia, Sudan and Yemen from the WHO Eastern Mediterranean Region, along with Eritrea, Ethiopia and South Sudan from the WHO African Region, and the Pakistan–Islamic Republic of Iran–Afghanistan Malaria Network (PIAM-Net). These networks aim to ensure continuous monitoring of the therapeutic efficacy of antimalarial medicines using updated WHO protocols. In most network countries, WHO supports activities and studies on drug efficacy testing at sentinel sites. In 2013, the gene for artemisinin resistance (K13 gene) was discovered and WHO was given the mandate to coordinate the tracking and mapping of the gene.

The sixth intercountry meeting of national malaria programme managers from the countries of HANMAT and PIAM-Net was organized by the WHO Regional Office for the Eastern Mediterranean in Cairo, Egypt, from 13 to 14 August 2014. The meeting aimed to disseminate information and update antimalarial drug monitoring mechanisms and treatment policies in countries. National malaria programme managers and focal points for case management attended from Afghanistan, Djibouti, Eritrea, Ethiopia, Islamic Republic of Iran, Pakistan, Saudi Arabia, Somalia, South Sudan and Sudan, as well as staff from the US Naval Medical Research Unit 3 (NAMRU-3). WHO staff from headquarters, regional and country levels also attended the meeting. The programme and list of participants are included as Annex 1 and 2, respectively.

2. UPDATE ON ARTEMISININ RESISTANCE AND MONITORING EFFICACY, AND PLANS FOR ITS CONTAINMENT

Dr M. Warsame, WHO headquarters

There are several tools available for monitoring drug efficacy and resistance, including in vivo studies using the WHO protocol (2009), pharmacokinetic studies, in vitro studies and studies of molecular markers. However, in vivo study results are the gold standard used to determine whether a change in treatment policy is required. Treatment failure in an individual patient is not always due to drug resistance, but may be due to other factors, including inadequate dosage, drugs of poor quality, pharmacokinetic factors, and patient immunity and non-compliance. Polymerase chain reaction (PCR) analysis must be conducted on samples from patients with treatment failure to determine whether treatment failure during follow-up was due to true recrudescence or re-infection.

Regular monitoring is needed for early detection of changes in treatment efficacy. Consistent monitoring every two years at the same sentinel sites, according to WHO protocol, allows for analysis of trends over time. The early detection of artemisinin resistance in Cambodia

was achieved when delayed parasite clearance was first observed in Pailin province. Recent studies have determined that artemisinin resistance is likely to be spreading and emerging independently, making "containment" of the problem more complex. Currently, artemisinin resistance is limited to the South-East Asia Region. The risk of further spread and emergence of artemisinin resistance can be reduced by scaling-up diagnostic testing, removing monotherapies and sub-medicines that do not meet quality standards from global markets and following the recommendations outlined in the Global plan for artemisinin resistance containment.

3. COUNTRY PRESENTATIONS OF THERAPEUTIC EFFICACY STUDY RESULTS

3.1 Afghanistan

The number of confirmed cases for both *Plasmodium falciparum* and *P. vivax* malaria has steadily decreased from 2002 to 2013. Since 2003, therapeutic efficacy studies (TES) have been conducted in three sentinel sites in Faryab, Nangarhar and Takhar provinces. This year, the malaria control programme is monitoring the efficacy of artemether-lumefantrine (AL) for the treatment of uncomplicated *P. falciparum* and *P. vivax* in four health facilities in Nangarhar and Kunar provinces. The study, which targets a sample of 100 patients each with *P. falciparum* and *P. vivax*, will conclude in November 2014.

3.2 Djibouti

In 2013, artesunate + sulphadoxine-pyrimethamine (AS+SP) was removed from Djibouti's national treatment policy due to concerns about possible resistance to SP**.** Challenges faced by the Djibouti national malaria control programme include: government procured treatments without proof of quality control; patient preference for treatments despite negative diagnostic results; patient preference for injected medicine over tablets. Djibouti has recently replenished their supply of medicine and rapid diagnostic tests for both species).

3.3 Eritrea

TES on artemisinin-based combination therapies (ACTs) have demonstrated high cure rates (> 90%) in Eritrea since 2006. Data from the most recent studies (2012/2013) conducted on artesunate + amodiaquine (AS+AQ) are available from four sentinel sites in the Gash Barka region. PCR-corrected treatment efficacy was found to be above 90% in all sites (range: 92.9–100%). The Eritrea national malaria control programme plans to conduct studies in more sites and is planning to conduct studies on *P. vivax*. However, the declining incidence of malaria is making it difficult to achieve the required sample sizes.

3.4 Ethiopia

TES results were presented from 2003 onwards. Between 2010 and 2012, studies were conducted on AL in seven sentinel sites. The average adequate clinical and parasitological response (ACPR) for this combination was 99% (range: 96.7–100%). In 2013, the PCR-corrected cure rates of treatment with AL remained high, at 99% and 100%. The national

malaria control programme was advised to test the efficacy of medicines needed to treat *P. vivax*, in particular AL.

3.5 Islamic Republic of Iran

The Islamic Republic of Iran is currently implementing a national malaria elimination plan, with the goal of elimination by 2025. There has been a marked reduction in malaria incidence, for both *P. falciparum* and *P. vivax,* over recent years. The most recent TES (2013) of AS+SP had an ACPR of 100%. It was recognized that the caseload in the country is heavily influenced by the neighbouring countries of Afghanistan and Pakistan. Challenges for the national malaria control programme include population movements and security at eastern border areas and the detection and follow-up of imported cases. Currently, all detected cases are reported and tracked through the public health system.

3.6 Pakistan

Pakistan has an annual caseload of 300 000 confirmed cases and an estimated 4.5 million cases of clinical (unconfirmed) malaria, most of which are from Punjab province, and many of which are not likely to be malaria. Currently there are four sentinel sites in place for TES. There are several challenges, including an unregulated private sector, inappropriate malaria treatment, with misuse of injection for uncomplicated malaria in the private sector, and control of malaria in border districts, with mass population movement from endemic to non-endemic areas. Selected achievements include increased coverage of diagnosis, provision of treatment to all confirmed cases, activities to increase public awareness on diagnosis and treatment, and partnership building with academia.

3.7 Saudi Arabia

Saudi Arabia is currently engaging in a national malaria elimination programme, which has lead to a significant drop in malaria incidence. Challenges faced by the national malaria control programme include follow-up for patients who are not residents (undocumented migrants) and the conduct of research by some academic institutions without coordination with the national programme.

3.8 Somalia

Currently there are three sentinel sites in Somalia (in Jamame, Janale and Jowhar), with a fourth planned for 2014 (in Bosasso). In 2011, the PCR-corrected ACPRs in the three sites monitoring AS+SP were 77.8% in Jamame, 99% in Jowhar and 95.6% in Janale. Quadruple and quintuple molecular markers conferring resistance to SP were found in Jamame (60% of samples) and Jowhar (40% of samples). In 2013, TES of AL conducted in Janale and Jowhar found close to 100% ACPR in both sites. Somalia is the first country in the Eastern Mediterranean Region to demonstrate resistance to SP. The national programme has been encouraged to take further steps to update the treatment policy with an effective medicine and to examine the value of using SP for intermittent preventive therapy. It is important that the

malaria indicator survey should be used as the key source of data for making estimates about malaria prevalence.

3.9 South Sudan

From August to December 2013, South Sudan conducted TES on AS+AQ and AL (28 days) at three health care centres (in Rejaf, Rubkona and Mapel). Among the 93 cases recruited at one site, 58 patients completed the study. The study is currently on hold and the data has not yet been analysed. Currently, no efficacy data is available on AL, the second-line treatment. The 2013 studies were the first TES to be conducted since 2004, when studies of AS+SP and AS+AQ determined a treatment efficacy of 91.2% and 92.7%, respectively. Many challenges for conducting TES in South Sudan exist, including lack of technical capacity within the country, insecurity leading to patient loss to follow-up, and slow and inadequate recruitment that prolongs study length and cost.

3.10 Sudan

In Sudan, sentinel sites have been established at five health centres. Studies were conducted on AS+SP and AL, according to the standard WHO protocol. Most studies conducted on AS+SP (2011–2012) showed a PCR-corrected ACPR above 95%. Studies of AL conducted in two sites in 2010 and 2012 also found a PCR-corrected ACPR above 95%. Data from studies conducted in 2013 have not yet been PCR-corrected; however, ACPR before PCR-correction is above 90%. Molecular analysis of dihydrofolate reductase *(dhfr)* and dihydropteroate synthase *(dhps)* gene mutations in the four sites found that few of the 356 patients had *dhfr* wild type (4%), while 84% had double *dhfr* and triple *dhfr* (11.5%). Triple mutations in the *dhps* gene were observed in all four sites, in particular Kassala (48%). One patient from Kassala was found to have a quadruple *dhps* mutation. When *dhfr/dhps* were analysed together, 23% of patients were found to have quintuple mutants and eight patients among the 352 had more than six mutations.

4. ISSUES RELATED TO UNIVERSAL COVERAGE OF DIAGNOSIS AND TREATMENT POLICY

Dr H. Atta, WHO Regional Office for the Eastern Mediterranean
Dr J. Nambose, WHO Office Zimbabwe

HANMAT/PIAM-Net countries should aim to ultimately confirm all suspected malaria cases. Confirmatory diagnostic tests not only help to refine true estimates of malaria, but also provide a clear benefit to those patients with non-malaria conditions, who can then receive appropriate treatment. Scale-up of confirmed diagnosis and phasing-out of clinical diagnosis can be facilitated with the use of rapid diagnostic tests at the community level. This is now even more feasible, given the low-cost of rapid diagnostic tests.

Treatment policies for each country were discussed, with the goal of harmonization across HANMAT/PIAM-Net countries. The outcomes of this discussion are reflected in the recommendations.

5. MOLECULAR MARKERS FOR THE DETECTION OF ANTIMALARIAL DRUG RESISTANCE

5.1 K13 marker of artemisinin resistance

Dr M. Warsame, WHO headquarters

Mutations in the Kelch propeller domain (K13 propeller) have been associated with artemisinin resistance, in vitro and in vivo. Specifically, the K13 mutation is associated with higher parasite survival rates in vitro and delayed parasite clearance in vivo. The mutant K13-propeller allele clusters in Cambodian provinces where resistance is prevalent, and the increasing frequency of a dominant mutant K13-propeller allele, correlates with the recent spread of resistance in western Cambodia. The K13 mutant has recently been observed to be present in studies conducted throughout mainland South-East Asia, from southern Viet Nam to central Myanmar, and the mutation is strongly associated with delayed parasite clearance. K13 propeller mutations therefore provide a means of detecting artemisinin resistance, and can be used to monitor the emergence and/or spread of artemisinin resistance globally.

The parasite clearance half-life (slope) is another tool for detecting artemisinin resistance and describes the time needed for parasitaemia to be reduced by half during the log linear phase of parasite clearance. A parasite clearance median slope of greater than or equal to five hours is considered delayed. Delayed parasite clearance has been observed in western Cambodia, Thailand and southern Myanmar. Delayed parasite clearance is associated with decreased parasite sensitivity to the artemisinin component, and is not likely to lead to treatment failure, provided the partner drug is effective.

A working definition of artemisinin resistance has been developed based on observations from routine therapeutic efficacy studies of ACTs, clinical trials of artesunate monotherapy and K13 sequencing. Suspected artemisinin resistance is defined as: ≥ 5% of patients carrying K13 resistance-associated mutations; or ≥ 10% of patients with persistent parasitaemia by microscopy on day 3 after treatment with ACT or artesunate monotherapy; or ≥ 10% of patients with a parasite clearance half-life ≥ 5 hours after treatment with ACT or artesunate monotherapy.

Confirmed artemisinin resistance is defined as: ≥ 5% of patients carrying K13 resistance-associated mutations, all of whom have been found, after treatment with ACT or artesunate monotherapy, to have either persistent parasitaemia by microscopy on day 3, or a parasite clearance half-life of ≥ 5 hours. Confirmed artemisinin resistance (with molecular markers) should prompt the initiation of containment activities. Countries are encouraged to collect an extra filter paper blood spot on day 0 for the detection of K13 mutations. Countries are also encouraged to report the percentage of patients with parasitaemia on day 3 at future network meetings, as well as in reports and publications.

The definition of ACT resistance has also been refined. ACT resistance is now defined as: the presence of artemisinin resistance as defined above; and the presence of partner drug resistance = treatment failure + adequate blood level (if known) and/or presence of a validated molecular marker of resistance (if known). ACT failures > 10% should prompt a change in

policy. Treatment failures with or without day 3 will not systematically be considered ACT resistance as many factors can affect the efficacy of the partner drug in particular the drug absorption and metabolism

An expert group meeting will be organized by WHO in September 2014 to establish guidelines for surveillance of K13. Reference laboratories will need to be established, given the cost and complexity of the sequencing. NAMRU-3 has offered to analyse all pre-existing day 0 samples, following ethical clearance for the work from local ethics committees.

5.2 NAMRU-3 collaborative activities in genotyping to differentiate recrudescence from reinfection and in molecular marker studies

Dr G. Murpyh/Dr R. Abdelkhalek, NAMRU-3

Opportunities for collaboration with NAMRU-3 were presented and an overview provided the of techniques used for genotyping studies. NAMRU-3 is available for capacity-building (training and standard operating procedure writing) and molecular testing for identification of species, distinguishing between recrudescence and new infections, identification of molecular mechanisms of resistance, and determination of the country of origin of a given infection. The results of molecular studies using data collected from TES in Egypt, Jordan, Pakistan, Sudan and Yemen were presented. When countries send samples in the future, senders are encouraged to provide a clear indication of the day of failure, without which the analysis cannot be done.

5.3 The experience of the Pasteur Institute of Iran in molecular studies for monitoring markers of resistance to SP

Dr S. Zakeri, Pasteur Institute of Iran

In terms of molecular markers associated with resistance to antimalarial treatment, a distinction can be made between combinations of *dhfr/dhps* molecular mutations that are:

- partially resistant: *dhfr* (51I/59R/108N)/*dhps*(437G)
- fully resistant: *dhfr*(51I/59R/108N)/*dhps*(437G/540E)
- super-resistant: *dhfr*(51I/59R/108N/164L)/*dhps*(437G/540E/581G).

Combination therapy with an ineffective partner drug makes the artemisinin component vulnerable to resistance. All *dhfr*/*dhps* markers are needed in order to provide useful information on SP resistance. It is not advisable to predict SP resistance based on individual mutations. Furthermore, molecular markers are not always correlated with treatment outcome; therefore in vivo studies remain the gold standard for monitoring therapeutic efficacy.

Studies of the efficacy of chloroquine (CQ) for treatment of *P. vivax* are needed in the Islamic Republic of Iran. If necessary, an ACT may be used for treatment of *P. vivax*, following determination of efficacy through TES and molecular marker studies. Currently the Pasteur Institute of Iran is participating in the K13 artemisinin resistance multicentre rapid assessment (KARMA) project to describe the geographical distribution of K13 alleles in malaria-endemic countries and to map the prevalence of resistance.

5.4 Dihydroartemisinin-piperaquine for the treatment of *P. vivax* in southern Pakistan

Dr S. Shaikh

Interventions have brought a decline in *P. falciparum* over the last few years. Recent studies of AS+SP and AL have shown both treatments to be safe and effective for uncomplicated *P. falciparum* in Pakistan (ACPR of > 95%). Interventions have made less impact on the incidence of *P. vivax,* which has now become the dominant species. Policy recommendations for treatment of *P. vivax* are hindered by lack of information on the prevalence of glucose-6-phosphate dehydrogenase deficiency (G6PDd) and the efficacy of CQ. Primaquine (PQ) is not favoured by physicians and is not available on the private market.

The efficacy of dihydroartemisinin-piperaquine (DP) for the treatment of *P. vivax* was studied in Sindh province in 2013. The study was conducted according to the WHO protocol, with a follow-up of 42 days. The ACPR among patients who completed the study follow-up was 102/103 (99%). No adverse events were reported. Future studies are needed to determine the efficacy of DP for the treatment of *P. vivax* in other areas of Pakistan. In addition, if the efficacy of AL for *P. vivax* and DP for *P. falciparum* can be established, a single drug policy may be possible. Improved coverage of parasitological diagnosis is essential.

5.5 Studies of *P. falciparum* and *P. vivax* in Afghanistan

Dr G. Awab

In Afghanistan, clinical trials on the efficacy of DP and AS+SP for the treatment of *P. falciparum* were conducted in four sentinel sites (Jalalabad, Kunar, Takhar, Faryab) from 2007–2009, and more recently in Jalalabad (2010) and Kunar (2012–2013). Although in all studies, the ACPR remains high (95–100%), molecular mutations of resistance to SP have been observed. It is expected that without analysis of molecular markers, resistance to SP may have gone undetected, given that the artemisinin component is still effective. Analysis of *dhfr/dhps* mutations from the most recent studies is not yet available, but sequencing of the K13 gene has found all samples to be of the wild type.

P. vivax is one of the major causes of morbidity in Afghanistan. Studies of CQ and DP for the treatment of *P. vivax* conducted between 2007 and 2009 in Jalalabad, Taloqan and Maimana found an ACPR of 100% on day 28. Subsequent studies of CQ and CQ+PQ conducted between 2009 and 2013 also found high treatment efficacy (> 95% ACPR). A survey conducted to estimate the prevalence of G6PDd found an overall prevalence was 5.6% (n = 713). Since G6PDd is present in all major ethnic groups, testing must be conducted prior to treatment with PQ.

5.6 Mapping G6PD deficiency

Dr G. Zamani

Mapping of G6PDd is needed to ensure the safe use of PQ, a key medicine in malaria elimination. PQ is the only drug active against the dormant relapsing form of *P. vivax* and it is effective for treatment of *P. falciparum* gametocytes (which can help contain the spread of artemisinin resistance).

The Malaria Atlas Project provides spatial data on the prevalence of G6PDd in malaria-endemic areas. However, existing maps are limited: they do not show subnational data, effectively failing to reflect important differences in ethnic groups within regions. Estimates are often based on extrapolations from data collected in other countries and survey samples are not always representative. Further, prevalence estimates vary over time, not necessarily due to changes in prevalence but due to different methods of estimating.

Participants suggested that in order to complete the database and produce more defined estimates, a new tool is needed. Current tests are expensive. A standard WHO protocol on testing for G6PDd is also required to enable comparisons of estimates across endemic countries. In areas with high G6PDd prevalence, testing must be conducted prior to administration of PQ for radical treatment. It was recognized that in areas where the population is characterized by diverse ethnic groups, the map will be more complex. A grant from the Special Programme for Research and Training in Tropical Diseases (TDR) may be available next year to provide funds for this activity. A special G6PDd monitoring group may be established for the Eastern Mediterranean Region to determine how best to measure the prevalence of G6PDd in the Region.

6. RECOMMENDATIONS

To Member States

1. For the prevention of drug resistance, and in keeping with good clinical practice, countries should intensify their efforts to scale-up testing of suspected malaria cases before treatment with an antimalarial. Presumptive treatment of malaria should be phased out as soon as possible. Each country is encouraged to develop its own operational plan, with, appropriate targets and timelines, and submit this plan to the HANMAT/PIAM-Net networks. Resources should be secured from national or donor resources.

2. Countries of HANMAT and PIAM-Net should urgently review and update their antimalarial drug policy taking into consideration the following:
   - inclusion of single dose PQ as a gametocytocidal drug (for *P. falciparum*) for all Eastern Mediterranean Region countries and for Ethiopia and Eritrea in the African Region
   - inclusion of injectable artesunate as first option for treatment of severe malaria
   - inclusion of radical treatment of *P. vivax*/*P. ovale* with PQ (14 day or 8 week regimen)
   - indication of the treatment of mixed infections according to WHO guidelines.
3. Countries (especially Djibouti, Pakistan and Sudan) should take strong action and enforce regulations to ban the use of injectable artemether for the treatment of uncomplicated malaria. WHO country offices should follow up and update the Regional Office on action taken.

4. Countries should continue activities for monitoring in vivo efficacy of first- and second-line drugs and other potential medicines for treating *falciparum* malaria, and collect filter papers

for day 0 (preferably two papers) to perform molecular marker analysis of mutations associated with SP resistance as well as K13 mutations for artemisinin resistance.

5. Countries with a high proportion of *vivax* malaria should monitor the efficacy of CQ for *vivax* malaria and the efficacy of other potential alternatives.

To NAMRU-3

6. NAMRU-3 should continue its cooperation on molecular analysis. Subsequent to retrieving ethical clearance from local authorities, day 0 samples from 2013 studies can be analysed for K13 mutations.

To WHO

7. WHO Somalia, together with the national malaria control programme, should organize a meeting with all stakeholders for urgent review and update of the drug policy, in response to the 2011 study, that found high failure rates with AS+SP in Jamame and a high proportion of SP quintuple mutations in Jowhar and Jamame. The drug policy should consider the efficacy of AL observed during the 2013 study in Jowhar and Janale. The meeting should also review the policy of intermittent preventive treatment in pregnancy and its current value in Somalia, given the decrease in the prevalence observed during the malaria indicator survey in 2013.

8. WHO should develop a standard protocol to survey the prevalence of G6PDd and support research studies to update this information. A meeting should be conducted involving all partners working on mapping G6PDd, relevant researchers and programme staff in order to develop a joint action plan.

9. WHO should support countries to share the results of the drug efficacy studies in international journals, through support in developing manuscripts and publication. Collaboration with research institutes is also encouraged.

Annex 1

PROGRAMME

Wednesday, 13 August 2014		
08:00–08:30	Registration	
08:30–09:00	Opening Session	
	• Welcome note	*Dr J. Mahjour*
	• Nomination of officers	
	• Objectives of the meeting and methods of work	
09:00–09:30	Update on artemisinin resistance and monitoring efficacy, and plans for its containment	*Dr M. Warsame*
09:30–10:00	Discussion	
10:00–10:00	Treatment policies in Eastern Mediterranean Region countries	
	• Djibouti	
	• Saudi Arabia	
11:00–16:30	Country presentations on the results of therapeutic efficacy studies and current drug policies:	
	• Afghanistan	
	• Islamic Republic of Iran	
	• Pakistan	
	• Somalia	
	• South Sudan	
	• Sudan	
	• Yemen	
	• Eritrea	
	• Ethiopia	
16:30–17:30	Issues related to malaria drug policy relevant to network countries in both Eastern Mediterranean Region and African Region countries (update on treatment guidelines, drug availability, drug quality, dual drug policy, etc.)	*Moderators: Dr H. Atta, Dr J. Namboze*
Thursday, 14 August 2014		
08:30–09:00	K13 as a marker of artemisinin resistance	*Dr M. Warsame*
09:00–09:30	Discussion	
09:30–11:00	Use of molecular techniques in malaria	
	• NAMRU-3 collaborative activities in genotyping to differentiate recrudescence from reinfection and in molecular marker studies	*NAMRU-3*
	• Experience of the Pasteur Institute of Iran in molecular studies for monitoring markers of resistance to SP	*Dr S. Zakeri*
	• Discussion	
11:00–11:30	• Dihydroartemisinin-piperaquine for the treatment of *P. vivax* malaria in southern Pakistan	*Dr S. Shaikh*
	• Studies of *P. falciparum* and *P. vivax* in Afghanistan	*Dr G. Awab*
11:30–12:00	Mapping G6PD deficiency	*Dr G. Zamani*
12:00–16:30	Developing plans of the therapeutic efficacy studies for 2014–2015	
15:15–16:30	Conclusions and recommendations	
16:30–17:00	Closing session	

Annex 2

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