Review Article

Drug-resistant malaria in Sudan: A review of evidence and scenarios for the future

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ABSTRACT

Resistance of falciparum malaria to chloroquine (CQ) has gradually emerged in the late 1970s, reaching unacceptably high proportions over the following three decades of use as first line treatment in Sudan. By 2004-2006 CQ was replaced by artemisinin-based combination treatment (ACTs), with combination of sulfadoxine-pyrimethamine (SP) and artesunate (AS) deployed as first-line drug against falciparum malaria. The present review follows the evolution of CQ resistance in Sudan and the available evidence on the response to the present first-line drugs. The findings in Sudan are analyzed in view of developments in other African countries and at the global level, with the hope of elucidating possible scenarios for the course of events in the Sudan. Northern Sudan has been one of the areas where signals indicating the emergence of drug resistant malaria parasites have first originated in Africa. The pattern of low endemicity and low population immunity to malaria, together with massive deployment and improper use of anti-malarial drugs created the ideal environment for creation of anti-malarial drug resistance. Such an environment existed in certain areas in South East Asia that had historically been the epicenter from which falciparum malaria parasites resistant to pyrimethamine and chloroquine have spread to the rest of the world. The alarming recent reports about the emergence of artemisinin (ART) resistance in South East Asia have lead WHO to take specific measures for prevention, early detection   and containment of drug resistance. What could be applicable in Sudan in these measures is discussed here.

Key words: Malaria, drug resistance, Sudan, chloroquine, artemisinin.

INTRODUCTION

Malaria transmission in the Northern Sudan is low-to-moderate, highly seasonal and occasionally epidemic. In the northern Sudan, in 2010, there were 2398239 reported malaria cases and 1023 deaths in the states from which information is completely available, with more than 95% of the cases being due to Plasmodium falciparum [1]. Sudan is one of the African countries in which the emergence of chloroquine (CQ) resistant falciparum malaria in Africa was first reported. An in vivo study was
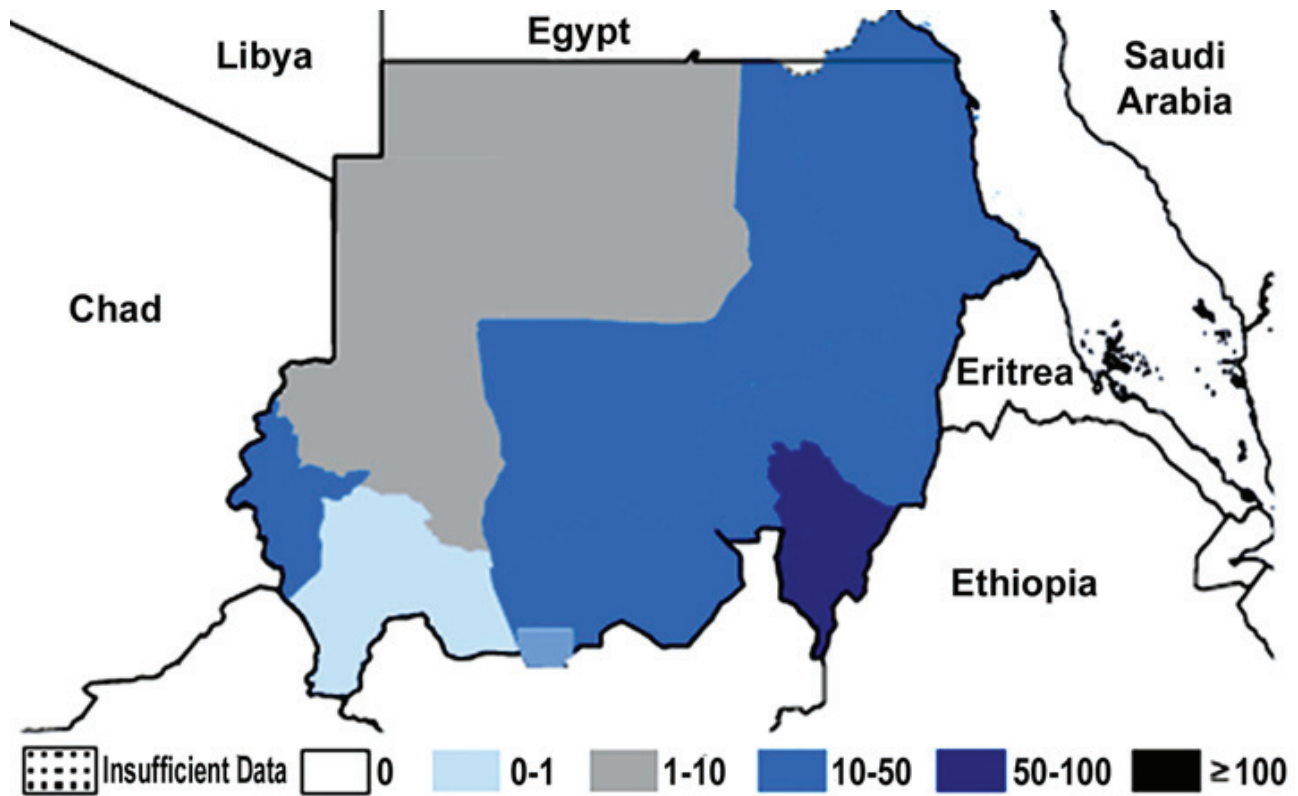

WHO, World Malaria Report, 2011 [1]

carried out in 1978, using the WHO standard in vivo test for 28 days in Gezira in Northern Sudan, and for 7 days in Bor in Southern Sudan [2]. Three cases of resistance were identified in Gezira but no cases were detected in Bor because the follow-up was done for only 7days in the Bor area. It is possible that cases with RI resistance could have been missed. In the same year a team from WHO conducted in vitro and in vivo studies in Sennar and reported that P. falciparum had lower sensitivity to CQ by the in vitro test than other African strains, and that parasite clearance time was slower by the in vivo test than in other African cases of malaria tested [3]. A definite confirmation of the extent of CQ resistance in Sudan was first demonstrated by an in vivo study in the Khartoum area. The study was done in 1982 in 8 villages about 25 km north of Khartoum. The test was carried out in 26 patients aged 4–20 years who were observed for 28 days. Ten were sensitive, 9 had RI and 7 had RII responses. It was also noted that parasite clearance was slower and more incomplete than in the previous studies

conducted in Sudan [4,5].

In the 1980s eastern Sudan faced a number of disasters, including drought, famine, floods, war and influx of refugees from Ethiopia. Under these conditions malaria outbreaks were frequent, with reports of substantial numbers of cases not responding to CQ. A study combining in vivo and in vitro assessment done in 1986 in eastern Sudan indicated 43% of P. falciparum infections investigated were resistant at RI, RII or RIII levels [6]. In another trial conducted in Gedaref State in 1987, an in vivo 28-day test for CQ efficacy was carried out on 63 patients and the response was reported as "Sensitive" (52%) , "R1" (18%) , "RII" (16%) and "RIII" (14%), no allowance was made for the possibility of re-infection. Blood level of CQ was measured, ruling out sub-therapeutic levels as a cause for treatment failures [7]. In a hospital-based study reported in 1992 in vivo and in vitro studies were conducted in children with falciparum malaria in central Sudan, including measurement of plasma CQ levels. Among 52 cases

tested in this study, 25% were classified as RI-RIII in the in vivo test and sub-sample of 12 isolates tested in vitro indicated that 25% of the isolates did not show a satisfactory in vitro response [8].

Therapeutic efficacy tests involving CQ and based on post-1973 WHO protocols:

Therapeutic efficacy tests (TET) for anti-malarial drugs done in Sudan since 1996- were generally based on the contemporaneous published WHO protocols for therapeutic efficacy testing [9-12] and on unpublished drafts of these protocols that appeared in-between. Consequently, these studies show some minor variations in the methodology mainly in the areas of low transmission, regarding duration of the follow-up and the inclusion criteria.

The first reported study based on post-1973 WHO protocols was done in 1997/8 [13]. Falciparum malaria cases were recruited to 5 sentinel posts in northern and central Sudan with low malaria transmission. Sampling and data analysis were done by the lot quality assurance sampling (LQAS) method [14]. Based on this method prevalence of resistant strains were proven not to be significantly less than 25% at all sentinel posts. The high level of resistance (77%) in Kassala town (Kassala State) was attributed to population movements in the Eastern borders. The test in Dilling (Southern Kordofan State) also showed a high prevalence of resistance (72%) and this was suggested to be due to displacement of populations from Southern Sudan. One notable finding was that resistant cases in these low transmission areas were predominantly children, leading authors to suggest inclusion of children as a subgroup when testing efficacy in low transmission settings as they have a higher risk of therapeutic failure. At about the same time the Ministry of Health started a number of sentinel posts for monitoring therapeutic efficacy of CQ. Findings of these studies were documented in unpublished reports, [15], covering studies done during the period 1997-2002 and some of the results were presented in a regional

workshop [16]. Elamin et al [17] published studies done in two rounds of testing by the Ministry of Health in 2002/2003 , testing CQ, sulfadoxine-pyrimethamine (SP) and CQ +SP in several sentinel sites in the Northern Sudan reporting an overall failure rate of CQ arm as 43.7% in the Northern Sudan and 80.2% in the Southern Sudan. In New Halfa (Kassala State) Adam et al (a) [18] conducted a randomised trial in 1999-2000 including treatment CQ, Quinine and SP. CQ efficacy rate was found to be 23.1%.

In 2001, Van der Brook et al [19] tested amodiaquine (AQ), CQ and SP efficacy in Upper Nile State of Southern Sudan. CQ and AQ produced treatment failures of 11.5% and 5.6% respectively, with no statistical difference. In another location in Western Equatoria State of Southern Sudan, Stivanello et al [20] tested AQ, CQ and SP efficacy. The results showed high failure rates to CQ (93.9%) and SP (69.9%), and a lower failure rate for AQ (25.2%).

By 2003 the published and unpublished evidence for therapeutic failure of faciparum malaria in Sudan was reviewed and CQ failure rates greater than 25% were reported in 22 out of 25 therapeutic efficacy tests conducted in different sentinel posts in Sudan [21]. In 2004, Sudan changed its malaria treatment policy, replacing CQ with artemisinin-based combination therapy, Aretesunate +SP (AS/SP) was adopted as first line treatment against falciparum malaria and aremether - lumefantrine (AL) as second-line treatment [22]. ACTs were registered in Sudan in 2006 [1].

Therapeutic efficacy tests involving SP and based on post -1973 WHO protocols:

A pyrimidine derivative, proguanil, emerged from the anti-malarial pipeline during World War II [23]. Sulfones and sulfonamides were then combined with proguanil or pyrimethamine in hopes of increasing efficacy and forestalling or preventing resistance [24]. In spite of this, by 1955 P. falciparum resistant to pyrimethamine had already been noted in Sudan [25]. Soon after Sulfadoxine/

Pyrimethamine (SP) was introduced in Thailand in 1967, resistance appeared that same year and it spread quickly throughout South-East Asia. However SP maintained its efficacy in Africa until the late 1990s but since then its resistance has spread rapidly [26]. SP was introduced in the Sudan since the early 1970s- as second line treatment for cases of uncomplicated falciparum malaria. With adoption of AS-SP combination as first line treatment against falciparum malaria in Sudan, the interest in monitoring therapeutic efficacy of SP continues. SP resistant malaria was first reported in Sudan in 1991[27]. Table 1 shows the rates of treatment failures in reported

Table 1- In vivo studies reporting treatment failure with sulfadoxine-pyrimethamine (SP) as monotherapy and in combination with other anti-malarial drugs.

Author	Location	State	Year	SP	SP +CQ	SP +AQ	SP +AS	Reference
Adam 2004	New Halfa	Kassala	1999	0	ND	ND	ND	18
Adam 2004a	New Halfa	Kassala	2001	16.7	ND	ND	ND	28
Stivanello 2004	Mundri, Kajo Keji county	Western Equatoria	2001	69.1	ND	ND	ND	20
Van den broek 2003	Lankien	Upper Nile	2001	0	ND	ND	ND	19
A-Elbasit 2006	Darawish, Kajara	Gedaref	2003	31.7	36.6	ND	ND	29
Salah 2005	Kassala Town	Kassala	2003	15	2.5	ND	ND	30
Elamin 2007	Obeid	Northern Kordofan	2003	4.2	ND	ND	ND	17
Elamin 2007	Medani	Gezira	2003	2	ND	ND	ND	17
Elamin 2007	Kassala (Kassala state)	Kassala	2003	7.7	ND	ND	ND	17
Elamin 2007	Khartoum	Khartoum	2003	ND	14.5	ND	ND	17
Elamin 2007	Damazin	Blue Nile	2003	4	5.9	ND	ND	17
Hamour 2005	Limun village	Southern Kordofan	2003	ND	ND	7.2	8.8	33
Adam 2005	New Halfa	Kassala	2004	13.3	ND	ND	0	31
Mohamed 2006	Kosti	White Nile	2004	ND	ND	ND	0	36
Elamin 2005	Damazin	Blue Nile	2004	ND	ND	ND	0	37
Elamin 2005	Kassala Town	Kassala	2004	ND	ND	ND	4	37
Elamin 2005	Kosti	White Nile	2004	ND	ND	ND	0	37
Elamin 2005	Malakal	Upper Nile	2004	ND	ND	ND	0	37
Van Den Broek 2005	Malakal	Upper Nile	2003-4	ND	ND	1	0.9	39
Ibrahium 2007	New Halfa	Kassala	2005	ND	ND	4.8	2.5	32
Mukhtar 2007	Asar ,Abunaja Juama, Abunaja Bargo	Gedaref	2004-5	ND	ND	ND	15.6 (6.5)	38

AQ - amodiaquine, AS - artesunate, CQ - chloroquine, ND - not done, SP - sulfadoxine-pyrimethamine.

TETs evaluating SP as monotherapy or in combination with other anti-malarials. With the exception of two studies that reported no treatment failures with SP monotherapy in surveys conducted in 1999 in Kassala [18] and in 2001 in Upper Nile (Southern Sudan [19], other studies reported variable rates of treatment failures with SP monotherapy 17, 20,28,29,30,31].

SP in combination with CQ was then tested in Gedaref in Eastern Sudan where this combinations resulted in high treatment failure rates comparable to SP alone [29]. Combination of AQ+ SP resulted in low failure rates of 1% in Malakal (Upper Nile State), [39], and 4.8% New Halfa (Kassala State), [32] and 7.2% in Nuba Mountains in Southern Kordofan State [33, 34]. In conclusion, TETs on SP have provided evidence of emerging treatment failure with SP in Sudan. The emergence of SP resistance in Easter Sudan is confirmed by longitudinal observations with resistance markers which show a dramatic increase in the frequency of Pfdhfr 108N allele between 1998 and 2000 which is believed to reflect drug pressure due to increased SP usage during this period [35]. Five reported therapeutic efficacy tests conducted in 2003 -2004 for combination of SP +AS showed 100% efficacy of this combination against falciparum malaria in some sites in Northern and Southern Sudan whereas other sites show treatment failure rates ranging from 2 to 15.6 % in Northern and Southern Sudan. It is possible that efficacy of this combination is starting to be compromised by a growing resistance to the SP component as evidenced by 31.7 % failure rate of SP in Darawish in eastern Sudan [29].

Therapeutic efficacy tests involving other ACTs based on post 1973 WHO protocols:

Salah et al in 2005 [40], evaluated Artemether-Lumefantrine (AL) suspension amongst 48 children in Kassala in Eastern Sudan. All patients showed no clinical or parasitological failure by day 3. In Gedaref State, Mukthar et al 2007 [38] conducted TET study

in 3 villages for SP +AS and AL efficacy was 93.5% and 91.3% for SP +AS and AL respectively.

In 2003, the efficacies of mefloquine monotherapy and of an artesunate–mefloquine (AS+MQ) combination, against falciparum malaria, were investigated and compared in New Halfa, in eastern Sudan [41]. MQ was given at a dose of 25 mg/kg, AS+MQ at 4 mg/kg/day for 3 days followed by single-dose of MQ at 15 mg/kg. The cure rates were 92.5% after MQ and 94.7% after the AS+MQ combination. Although the treatments appeared equally effective in clearing parasitaemias, the combination was better at clearing gametocytaemias and was less likely to cause adverse side-effects. It is unclear why MQ given alone was almost 10-fold more likely to trigger adverse effects than treatment with a combination that contained the same drug. The authors suggest that this could be a reflection of the different mefloquine doses.

In 2010 Elamin et al [42] tested AL efficacy in 4 sentinel sites in Damazin (Blue Nile State), Sennar (Sennar State), Kosti (White Nile State) and Juba (Bah el-Jabal State). Although ETF and LCF were not excluded from the denominator in calculation of the proportion of treatment failures but figure for treatment failures still remained less than 5%.

Currently, TET in Northern Sudan is based in four sites in Kassala, Gezira, Blue Nile and White Nile States. Tests for artemisinin + piperaquine granules and tablets conducted in 2008 resulted in adequate clinical and parasitological response of 96.2% and 100 % respectively [43,44].

Studies on molecular markers of anti-malarial drug resistance in Sudan:

Studies on molecular markers in Sudan have mainly aimed at estimating the prevalence of the molecular markers of SP and CQ resistance and validation of the association of these markers with resistance in different locations for policy guidance as well for better understanding the evolution and epidemiology of drug resistance.

CQ resistance markers:

Studies on CQ resistance markers Pf CRT and Pf mdr1 have covered different parts of Northern Sudan and Southern Sudan [45-53, 55-65]. Cross-sectional studies have indicated geographic variations in the prevalence of these genes between sites in Eastern Sudan and central Sudan and the significant geographic differences have been attributed to differences in drug pressure [59]. Moreover longitudinal studies in one locality in eastern Sudan, where there are short and seasonal malaria epidemics, over the course of a 12-year period, indicated a steady increases in frequencies of the mutant alleles of pfcrt and pfmdr1 [35]. In addition, these studies revealed that these genes fluctuated significantly between the wet and the dry seasons, probably due to drug pressure. In spite of the fact that CQ has been abandoned as first line treatment for falciparum malaria, recent surveys show that the CQ resistance markers are still highly prevalent in endemic areas in Central and Eastern Sudan [56].

The correlation between drug resistance markers and treatment failure has recently been studied at the global level in a metanalysis of published studies [54]. This analysis concluded that the risk of therapeutic failure after CQ was increased by the presence of Pfcrt K76T and that Pfmdr1 N86Y was associated with both CQ and AQ failures. In Sudan, reports on the association of CQ resistance markers with response to treatment with anti-malarials show some discrepancies. Menegon et al [56] tested the correlation between in vitro susceptibility to CQ and the presence of single and double mutations Pfcrt T 76 / Pfmdr1 Y 86 of CQ in a sample of 45 isolates from Central and Eastern Sudan. They reported a low correlation between molecular profile of both genes and their in vitro susceptibility. This has not been fully explained but could be attributed to the small number in the test sample of that particular study. In fact, Sudanese isolates of P. falciparum lacking Pfmdr1 were first reported in 1991 [60]. The chemotherapeutic model also shows variable results. In an area of stable transmission in the Southern Sudan a strong association between CQ resistance markers and treatment failure was reported [57]. However, studies in areas of low transmission in Sudan found variable patterns of association between CQ treatment failure and polymorphisms pfcrt K76T and pfmdr1N86Y. Some studies found no association of these markers with clinical outcome [48, 53]. Gadalla [50] found a strong association of these markers with early treatment failure but not with the overall treatment response with CQ. Babiker et al [45] found significant association of treatment failure with pfcrt T76 polymorphism but association with pfmdr1 Y86 only in clones with pfcrt-T76, suggesting that pfmdr1 –Y86 augments drug resistance but only when pfcrt-76 is found in the same genome. Khalil et al [47] compared the association of CQ markers with response to treatment in Sudan and Tanzania. They found significant association between resistance markers and treatment failure in Sudanese isolates but not in isolates from Tanzania. They suggest that most of these discrepancies in the in vivo therapeutic model are probably related to host factors such as immunity and the parasite biomass at the start of treatment.

SP resistance markers:

Although SP is no longer recommended as monotherapy against falciraum malaria, it is still being used in combination with artesunate as first line treatment in Sudan as well as in other countries in the region. It follows that efficacy of the SP component should be closely monitored to ensure the effectiveness of the combination therapies that include it. The prevalence of point mutations Pfdhfr and Pfdhps have been reported from different parts of Sudan. A notable feature is that the latter surveys show high frequencies of mutations S108N and N51I either as individual mutations or as a combined double mutation in areas of low transmission in central and eastern Sudan.

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This allele of P. falciparum parasites in eastern Sudan has been associated with in vitro resistance to pyimethamine [46, 67], and has shown an increase in prevalence in the eastern Sudan corresponding to increased SP failure. Surveys conducted in Sudan also indicate low frequency of mutant IRN-GE haplotype which is commonly indicated as a good predictor of treatment failure [68]. Also, mutation Pfdhfr C59R which is also a good predictor of treatment failure [80,81], is absent or is very rare is Sudan. However, the frequency of this mutation in Sudanese isolates has significantly increased from 0. 6% in 2002-3 to 8.8% in 2007 in areas of low transmission suggesting emergence of SP resistance [56]. Al-Saai et al [65] described resistant haplotypes of Pfdhfr and Pfdhps in Asar in eastern Sudan distinct compared to common haplotypes thought to be the sources of resistance to SP in other African countries[73-79]. They conducted microsatellite studies of these haplotypes and the local genotypes suggested that resistant haplotypes in this area may have evolved locally.

The relationship between SP molecular markers and parasite clearance after treatment is rather complex. This relationship has been studied by A- Elbasit et al [49] who concluded that there are at least three factors directly contributing to parasite clearance: the drug (SP), immunity (represented by age) and parasite mutations (more than one mutation). They suggested that, the dhfr/dhps mutations provide the parasite the ability to resist chemotherapy, while it renders it more susceptible to the host immunity.

Molecular marker screening should be carried out in the frame of country surveillance activities. Monitoring evolution of P. falciparum drug resistance by analyzing the prevalence of drug resistance mutations in an endemic area as Sudan, where both logistical and political problems make the implementation of the therapeutic efficacy tests difficult, could provide key information for the deployment of an effective national malaria treatment drug policy.

5: The threat of emergence of artemisisnin resistance:

Recently, partial artemisinin-resistant falciparum malaria has emerged on the Cambodia–Thailand border [82, 83, 84]. The first signals came from the Thai Cambodian border when the authorities noted that routine surveillance data indicated a progressive failure of ACTs against falciparum malaria. Comprehensive studies with artesunate combined with mefloquine and as monotherapy have confirmed significant delay of parasite clearance in this area. In some cases there was evidence resistance manifesting as treatment failure. Several factors may have contributed to the emergence of reduced artemisinin sensitivity in Cambodia which was one of the first countries to adopt ACTs as a first-line treatment in 2001, but unregulated artemisininsin combination or as a monotherapy has been available since the mid-1970s. Counterfeited or substandard tablets that contain less active ingredients than stated are additional sources of subtherapeutic dosing of artemisinins, which may also have contributed to the selection of resistant parasite strains. It is possible that the different pharmacokinetic properties of artemisinins in subgroups of the population, such as pregnant women and children, have resulted in under-dosing. Why has this problem emerged in western Cambodia and not in other parts of the world? Several factors have probably played a role:

1. The aremisinins have been used for a long time (30 years) in this area, whereas most other countries have a shorter experience with these drugs.

2. There has been unique massive drug pressure.

3. The low malaria transmission in the area was probably essential to allow resistant parasite populations to establish themselves.

4. It is also possible that parasite factors, such as a unique P. falciparum phenotype or host factors have played a part. Western Cambodia has previously been a focal point for the emergence

of chloroquine resistance and for sulfadoxin–pyrimethamine resistance.

At some time resistance to the artemisinins was not considered to be a significant threat based on what was widely circulated about its use for centuries in China, without emergence of resistance. Now we know the fact that resistance to the artemisinins does happen .Yet, it is important to stress that the emerging resistance to artemisinins is still limited and will remain geographically limited for some time. However, judging from the history of anti-malarial drugs, this resistance is bound to reach Africa. That would be a real challenge since we still have no replacement for the artemisinins. A multifaceted containment programme has recently been launched by WHO in a programme called Global Plan for Artemisinin Resistance Containment (GPARC) [85]. The plan aims at prevention, early detection and containment of resistance to the aremisinins, with the goal of containment or elimination of artemisinin resistance where it already exists and prevents artemisinin resistance where it has not yet appeared.

It is apparent that the Sudan harbours the environment for creation of drug resistant malaria parasites as evidenced by the early and rapid spread of resistance to CQ. Besides the massive use of anti-malarials, there is a pattern of low endemicity which has been associated with spread of drug resistant malaria as shown by mathematical models [86]. The current threat of resistance to artemisinin should call for promotion of early diagnosis and appropriate treatment, decreasing drug pressure, optimising vector control, targeting the mobile population, strengthening management and surveillance systems, and operational research.

Therapeutic efficacy trials recently conducted in Sudan have provided valuable evidence to guide the country' national treatment policy. An even closer monitoring of the response to the present first line treatment (sulfadoxine-pyrimethamine plus artesunate) is needed. Of particular concern are the results of monitoring studies indicating treatment failure of first line treatment ACTs in eastern Sudan [38]. There is also evidence of increasing prevalence of markers suggesting resistance to the antifolates in the Sudan [56]. This raises concern since failure of SP as a partners drug in aremisinin-based combination would lead to failure of treatment with ACT and exposes artesunate to drug pressure leading to emergence of resistance to the artemisinins. In terms of practical steps to be taken we need to do the following:

(1) Monitoring response to anti-malarials:

We need to conduct regular rounds of monitor drug efficacy using the standard in vivo efficacy study protocol with ACT. If >10% positive after 72 hours, this could be an indication that we need further studies. It is important to note that treatment failure with ACT does not necessarily mean artemisinin resistance. To confirm the possibility of ART resistance we need to conduct a special study with ART monotherapy for 7 days , with the following additional monitoring parameters:

1. Parasite positivity at day 3 after treatment
2. Pharmacokinetc measurements
3. Parasite clearance time
4. PRR (Parasite reduction ratio) in 48 hours
5. Slope of the linear parasite clearance curve

(2) Taking measures to prevent or delay emergence of resistance:

This could be achieved by making effective ACTs of good quality widely accessible and correctly used, particularly in the private sector, which includes:

1. Education of the practitioners
2. Increase compliance by users of ACTs
3. Better diagnosis of the disease to avoid misuse of the medicines
4. Transmission control to reduce the burden and the use of anti-malarial drugs (less drug pressure) vector control and bed-nets

5. Reduction of reservoir of infection (responsible for the spread of drug resistance) in improving therapeutic practice, in particular early diagnosis, effective treatment, and use of gametocytocidal drugs

At one time resistance to the artemisinins was not considered to be a significant threat based on what was widely circulated about how it has maintained potency against malaria parasites for centuries in China. Now we know the fact that resistance to the artemisinins does happen although it has so far been confirmed in a small number of individual cases in a limited geographic area. There is no evidence now that ART resistance has spread to Africa but, judging from the history of anti-malarial drugs, this resistance is bound to reach Africa. It is just a matter of time.

REFERENCES

1. World Malaria Report 2011, Country Profiles, Sudan. At: http://www.who.int/malaria/world_malaria_report_2011/en/
2. Omer AHS. Response of Plasmodium falciparum in Sudan to chloroquine. American Journal of Tropical Medicine and Hygiene, 1978; 27:853–7.
3. Kouznetsov RL Rooney W, Wernsdorfer WH, El Gaddal AA, Payne D, and Abdalla RE. Use of the in vitro microtechnique for the assessment of drug sensitivity of Plasmodium falciparum in Sennar, Sudan. Bulletin of the World Health Organization, 1980, 58(5):785–9.
4. Al-Tawil N, Akood MA. Response of falciparum malaria to standard regimen of chloroquine in Khartoum Province, Sudan (unpublished document).WHO/MAL.83.991 (1983).
5. Al-Tawil N, Akood MA. Response of falciparum malaria to standard regimen of chloroquine in Khartoum Province, Sudan. East African Medical Journal,1983; 60:663–8.
6. Bayoumi RA , Babiker HA, Ibrahim SM et al. Chloroquine resistant Plasmodium falciparum in eastern Sudan. Acta tropica, 1989; 46(3):157–65.
7. Saeed BO, M A Hassabalrasoul, K E Ibrahim et al. Plasma chloroquine measurement in the evaluation of Plasmodium falciparum sensitivity. Journal of Tropical Medicine and Hygiene,1990;93:(6):393–6.
8. Ibrahim AM, Ali FR, Ali ME. Assessment of chloroquine resistance of Plasmodium falciparum in children of Wad Medani (Central Sudan). Journal of Tropical Pediatrics, 1992; 38(4):162–6.
9. Chemotherapy of malaria and resistance to anti-malarials. Geneva, World Health Organization, 1973 (WHO Technical Report Series, No. 529).
10. Assessment of therapeutic efficacy of anti-malarial drugs for uncomplicated falciparum malaria in areas with intense transmission. Geneva, World Health Organization,1996. (MAL/96.1077).
11. Assessment and monitoring of anti-malarial drug efficacy for the treatment of uncomplicated falciparum malaria. Geneva, World Health Organization, 2003, (WHO/HTM/RBM/2003.50).
12. Methods for surveillance of anti-malarial drug efficacy.World Health Organization.2009
13. Abdel–Hameed AA, El–Jak IE, Faragalla IA. Sentinel posts for monitoring therapeutic efficacy of anti-malarial drugs against Plasmodium falciparum in the Sudan. African Journal of Medicine and Medical Sciences, 2001; 30(S):1–5.
14. Lemeshow S and Taber S. Lot quality assurance sampling: single and double-sampling plans. World Health Statistics Quarterly 1991;44(3) : 115-132.
15. Final reports from sentinel sites. National Malaria Control Programme, National Malaria, Schistosomiasis and Leishmaniasis Administration. Khartoum, Federal Ministry of Health, 1997–2002.
16. Report on the Intercountry Workshop on Monitoring Therpeutic Efficacy of Anti-malarial Drugs .Sana'a, Republic of Yemen, 21–25 April 2002, WHO-EM/MAL/281/E/L.
17. Elamin SB, El-F.M. Malik, El-D.S. Ahmed, E.K. ElAbadi and T.A. Mohamad. Efficacy of chloroquine and sulfadoxine/pyrimethamine mono- and combined therapy against falciparum malaria in Sudan. East Mediterranean Health Journal . 2007;13:25-34.
18. Adam I, Osman ME, Elghzali G, Ahmed GI, Gustafssons LL, Elbashir MI. Efficacies of chloroquine, sulphadoxinepyrimethamine and quinine for the treatment of uncomplicated Plasmodium falciparum malaria in Eastern Sudan. Annals of Tropical Medicine and Parasitology 2004;98:661–666
19. Van den Broek IV, Gatkoi T, Lowoko B, Nzila A, Ochong E, Keus K. Chloroquine, sulfadoxine-pyrimethamine and amodiaquine efficacy for the treatment of uncomplicated Plasmodium falciparum malaria in Upper Nile, south

Sudan. Transactions of the Royal Society of Tropical Medicine and Hygiene 2003;97:229–235

20. Stivanello E, Cavailler P, Cassano F, et al. Efficacy of chloroquine, sulphadoxine–pyrimethamine and amodiaquine for treatment of uncomplicated Plasmodium falciparum malaria in Kajo Keji county, Sudan. Tropical Medicine and International Health. 2004;9:975-980

21. Resistance in Sudan and Africa , presentation of studies and results I. Resistance in Sudan page 20-22 In :Treatment options against malaria in Sudan. Conference report .14-15 October 2003.Khartoum, Sudan .Federal Ministry of Health, World Health Organisation , Médecins Sans Frontières. www.emro.who.int/rbm/SudanConference1003.pdf

22. Malik EM , Mohamed TA, Elmardi KA,et al. From chloroquine to artemisinin-based combination therapy: the Sudanese experience. Malaria Journal 2006;5:65

23. Greenwood D. Conflicts of interest: the genesis of synthetic anti-malarial agents in peace and war. Journal of Antimicrobial Chemotherapy .1995 ;36(5):857-72.

24. Kenneth J et al, (Editors) Saving Lives, Buying Time: Economics of Malaria Drugs in an Age of Resistance (2004) Institute of medicine of the National Academies,The National Academies Press NATIONAL ACADEMIES PRESS, 2004 Washington, D.C. http://www.nap.edu/catalog.php?record_id=11017

25. Abbot PH . Trials with Camoquine and Daraprim in treqatment of malaria at Wad Medani , Sudan . Journal of the Sudan Medical Association 1955; 3:33-38.

26. Mita T, Tanabe K , and K Kita Spread and evolution of Plasmodium falciparum drug resistance . Parasitology International 2009; 58:201-209.

27. Ibrahim ME, Awad-el-Kariem FM, el Hassan IM, el Mubarak ER. A case of Plasmodium falciparum malaria sensitive to chloroquine but resistant to pyrimethamine/sulfadoxine in Sennar,Sudan. Transactions of the Royal Society of Tropical Medicine and Hygiene 1991; 85: 446.

28. Adam I, Ibrahim MH, A/elbasit IA, Elbashir MI .Efficacy of sulfadoxine- pyrimethamine for uncomplicated Plasmodium falciparum malaria in a small sample of Sudanese children. East Mediterranean Health Journal 2004; 10:309–314

29. 29 A-Elbasit IE , Elbashir MI, Khalil IF, Alifrangis M, Giha HA. The efficacy of sulfadoxine-pyrimethamine alone and in combination with chloroquine for malaria treatment in rural eastern Sudan: the interrelation between resistance, age and gametocytogenesis . Tropical Medicine and International Health 2006; 11: 604 – 612

30. Salah MT, Mohammed MM, Himeidan YE, Malik EM, Elbashir MI, Adam I. A randomized comparison of sulphadoxine pyrimethamine and combination of sulphadoxine pyrimethamine with chloroquine in the treatment of uncomplicated falciparum malaria in Eastern Sudan. Saudi Medical Journal 2005; 26:147–148.

31. Adam I, A-Elbasit IE, Idris SM, Malik EM, Elbashir MI. A comparison of the efficacy of artesunate plus sulfadoxinepyrimethamine with that of sulfadoxine-pyrimethamine alone, in the treatment of uncomplicated, Plasmodium falciparum malaria in eastern Sudan. Annals of Tropical Medicine and Parasitology 2005; 9:449–455

32. Ibrahium AM, Kheir MM, Osman ME, et al. Efficacies of artesunate plus either sulfadoxine–pyrimethamine or amodiaquine, for the treatment of uncomplicated, Plasmodium falciparum malaria in eastern Sudan. Annals of Tropical Medicine and Parasitology 2007;101(1):15-21

33. Hamour S, Melaku Y, Keus K, et al Malaria in the Nuba Mountains of Sudan: baseline genotypic resistance and efficacy of the artesunate plus sulfadoxine-pyrimethamine and artesunate plus amodiaquine combinations. Transactions of the Royal Society of Tropical Medicine and Hygiene 2005;99:548–554

34. Yusuf RU, Omar SA, Ngure RM. The effect of point mutations in dihydrofolate reductase genes and multidrug resistance gene 1-86 on treatment of falciparum malaria in Sudan. J Infect Dev Ctries. 2010 ;4:61-9.

35. Abdel-Muhsin AM, Mackinnon MJ, Ali E, et al. Evolution of drug-resistance genes in Plasmodium falciparum in an area of seasonal malaria transmission in Eastern Sudan. Journal of Infectious Diseases 2004;189 :1239-44.

36. Mohamed AO, Eltaib EH, Ahmed OA, Elamin SB, Malik EM. The efficacies of artesunate-sulfadoxine-pyrimethamine and artemether-lumefantrine in the treatment of uncomplicated Plasmodium falciparum malaria, in an area of low transmission in central Sudan. Annals of Tropical Medicine and Parasitology 2006; 100:5–10

37. Elamin SB, Malik EM, Abdelgadir T, et al , Artesunate plus sulfadoxinepyrimethamine for treatment of uncomplicated Plasmodium falciparum malaria in Sudan. Malaria Journal 2005; 4:41

38. Mukhtar EA, Gadalla NB, El-Zaki SE, et al. A comparative study on the efficacy of artesunate plus sulphadoxine/ pyrimethamine versus artemether-lumefantrine in eastern Sudan. Malaria Journal,2007; 6:92.

39. Van den Broek I, Amsalu R, Balasegaram M, et al. Efficacy of two artemisinin combination therapies for uncomplicated falciparum malaria in children under 5 years, Malakal, Upper Nile, Sudan. Malaria Journal 2005; 4:14

40. Salah M T, Faroug M, Magzoub MM, Adam I. Efficacy of artemether-lumefantrine (Co-Artesiane®) suspension in the treatment of uncomplicated Plasmodium falciparum malaria among children under 5 years in eastern Sudan. Trop

J Pharm Res 2006;5(1):551–555

41. Adam I, A-Elbasit IE, Elbashir MI. Efficacies of mefloquine alone and of artesunate followed by mefloquine, for the treatment of uncomplicated, Plasmodium falciparum malaria in eastern Sudan. Annals of Tropical Medicine and Parasitology 2005; 99:111–117

42. Elamin B A , Awad AI, Eltayeb IB, et al . Descriptive study on the efficacy of artemether-lumefantrine in the treatment of uncomplicated Plasmodium falciparum malaria in Sudan. Eur J Clin Pharmacol 2010; 66:231-237

43. Report on the use of serological techniques in malaria epidemiology and management for HANMAT countries. Alexandria , Egypt 2-4 June , 2009,W-EM/MAL/335/E.

44. Intercountry Meeting for Members of HANMAT and PIAM-NET, 25 - 26 September 2010, Marrakech, Morocco.

45. Babiker HA, Pringle SJ, Abdel-Muhsin A, Mackinnon M, Hunt P, Walliker D. High-level chloroquine resistance in Sudanese isolates of Plasmodium falciparum is associated with mutations in the chloroquine sistance transporter gene pfcrt and the multidrug resistance Gene pfmdr1. Journal of Infectious Diseases 2001; 183:1535–1538

46. Babiker HA, Creasey AM, Bayoumi RA, Walliker D, Arnot D. Genetic diversity of Plasmodium falciparum in a village in eastern Sudan. 2. Drug resistance, molecular karyotypes and the mdr1 genotype of recent isolates. Transactions of the Royal Society of Tropical Medicine and Hygiene. 1991; 85:578-83.

47. Khalil IF, Alifrangis M, Tarimo DS, et al. The roles of the pfcrt 76T and pfmdr1 86Y mutations, immunity and the initial level of parasitaemia, in predicting the outcome of chloroquine treatment in two areas with different transmission intensities. Annals of Tropical Medicine and Parasitology 2005 ;99:441-448.

48. Tagelsir N, Ibrahim Z, Medani A, et al. High frequency of Plasmodium falciparum PfCRT K76T and PfpghN86Y in patients clearing infection after chloroquine treatment in the Sudan. Acta Tropica,2006 ; 97:19-25

49. A-Elbasit I , Alifrangis M, Khalil IF, et al. The implication of dihydrofolate reductase and dihydropteroate synthetase gene mutations in modification of Plasmodium falciparum characteristics .Malaria Journal 2007; 6:108.

50. Gadalla NB, lzaki SE, Mukhtar E, Warhurst DC, El-Sayed B, Sutherland CJ.Dynamics of pfcrt alleles CVMNK and CVIET in chloroquine-treated Sudanese patients infected with Plasmodium falciparum .Malaria Journal 2010 ; 12;9:74.

51. Giha HA, Elbashir MI, A-Elbasit IE, et al. Drug resistance-virulence elationship in Plasmodium falciparum causing severe malaria in an area of seasonal and unstable transmission. Acta Tropica. 2006 ;97:181-7.

52. Ali E, Mackinnon MJ, Abdel-Muhsin AM, Ahmed S, Walliker D, Babiker HA. Increased density but not prevalence of gametocytes following drug treatment of Plasmodium falciparum. Transactions of the Royal Society of Tropical Medicine and Hygiene. 2006 ;100(2):176-83.

53. A-Elbasit IE, Khalil IF, Elbashir MI, et al. High frequency of Plasmodium falciparum CICNI/SGEAA and CVIET haplotypes without association with resistance to sulfadoxine/pyrimethamine and chloroquine combination in the Daraweesh area, in Sudan. Eur J Clin Microbiol Infect Dis. 2008 ;27(8):725-32

54. Stéphane P, Olliaro P, de Monbrison F, Bienvenu AL, Price RN, Ringwald P. A systematic review and meta-analysis of evidence for correlation between molecular markers of parasite resistance and treatment outcome in falciparum malaria. Malaria Journal 2009; 8:89

55. Osman ME , Mockenhaupt, Mockenhaupt FP, Bienzle U, Elbashir MI, Giha HA. Field-based evidence for linkage of mutations associated with chloroquine (pfcrt/pfmdr1) and sulfadoxine-pyrimethamine (pfdhfr/pfdhps) resistance and for the fitness cost of multiple mutations in P. falciparum . Infect Genet Evol 2007;7: 52 – 59 .

56. Menegon M, Talha AA, Severini C, et al.Frequency distribution of anti-malarial drug resistance alleles among Plasmodium falciparum isolates from Gezira State, central Sudan, and Gedarif State, eastern Sudan. American Journal of Tropical Medicine and Hygiene. 2010 ;83:250-7.

57. Ochong EO, van den Broek IV, Keus K, Nzila A. Short report: association between chloroquine and amodiaquine resistance and allelic variation in the Plasmodium falciparum multiple drug resistance 1 gene and the chloroquine resistance transporter gene in isolates from the upper Nile in southern Sudan. American Journal of Tropical Medicine and Hygiene. 2003;69:184-7.

58. Anderson TJ , Nair S, Jacobzone C, Zavai A, Balkan S. Molecular assessment of drug resistance in Plasmodium falciparum from Bahr El Gazal province, Sudan. Tropical Medicine and International Health 2003; 8: 1068 – 1073 .

59. Abdel-Muhsin AA, Mackinnon MJ, Awadalla P, et al.Local differentiation in Plasmodium falciparum drug resistance genes in Sudan. .Parasitology. 2003;126(Pt 5):391-400.

60. Awad-El-Kariem, F.M., Miles, M.A., Warhurst, D.C. Chloroquine-resistant Plasmodium falciparum isolates from the Sudan lack two mutations in the Pfmdr-1 gene thought to be associated with Chloroquine resistance. Transactions of the Royal Society of Tropical Medicine and Hygiene 1992; 86: 587–589.

61. Khalil, IF, Alifrangis M, Rønn AM, et al. Pyrimethamine/sulfadoxine combination in the treatment of uncomplicated falciparum malaria: relation between dihydropteroate synthase/dihydrofolate reductase genotypes, sulfadoxine

plasma levels, and treatment outcome. American Journal of Tropical Medicine and Hygiene 2002; 67: 225–229.

62. Khalil I F , Rønn AM, Alifrangis M, Gabar HA, Satti GM, Bygbjerg IC. Dihydrofolate reductase and dihydropteroate synthase genotypes associated with in vitro resistance of Plasmodium falciparum to pyrimethamine, trimethoprim, sulfadoxine, and sulfamethoxazole . American Journal of Tropical Medicine and Hygiene 2003 ;68 :586-9.

63. Alifrangis M , Enosse S, Khalil IF, et al. Prediction of Plasmodium falciparum resistance to sulfadoxine/pyrimethamine in vivo by mutations in the dihydrofolate reductase and dihydropteroate synthetase genes: a comparative study between sites of differing endemicity . American Journal of Tropical Medicine and Hygiene 2003; 69: 601 – 606

64. Khalil IF , Rønn AM, Alifrangis M, et al. Response of Plasmodium falciparum to cotrimoxazole therapy: relationship with plasma drug concentrations and dihydrofolate reductase and dihydropteroate synthase genotypes. American Journal of Tropical Medicine and Hygiene 2005; 73:174-7.

65. Al-Saai S, Kheir A, Abdel-Muhsin AM, et al.Distinct haplotypes of dhfr and dhps among Plasmodium falciparum isolates in an area of high level of sulfadoxine-pyrimethamine (SP) resistance in eastern Sudan. Infect Genet Evol 2009; 9:778-83.

66. Plowe CV, Kublin JG, Doumbo OKP. falciparum dihydrofolate reductase and dihydropteroate synthase mutations: epidemiology and role in clinical resistance to antifolates. Drug Resist Updat 1998; 1 : 389–396.

67. Abdel-Muhsin AM, Ranford-Cartwright LC, Medani AR, et al. Detection of mutations in Plasmodium falciparum dihydrofolate reductase (dhfr) by dot-blot hybridisation. American Journal of Tropical Medicine and Hygiene 2002; 67: 24–27.

68. Kublin JG, Dzinjalamala FK, Kamwendo DD, et al. Molecular markers for failure of sulfadoxine-pyrimethamine and chlorproguanil-dapsone treatment of Plasmodium falciparum malaria. Journal of Infectious Diseases 2002; 185: 380–8.

69. Basco LK , Tahar R , Ringwald P. Molecular basis of in vivo resistance to sulfadoxine-pyrimethamine in African adult patients infected with Plasmodium falciparum malaria parasites . Antimicrobial Agents and Chemotherapy 1998; 42: 1811 – 1844 .

70. Cowman AF , M J Morry, B A Biggs, G A Cross, and S J Foote. Amino acid changes inked to pyrimethamine resistance in the dihydrofolate reductase-thymidylate synthase gene of Plasmodium falciparum . Proceedings of the National Academy of Sciences 2003;85: 9109 – 9113 .

71. De Pecoulas PE , Basco LK, Le Bras J, Mazabraud A. Association between antifol resistance in vitro and DHFR gene point mutation in Plasmodium falciparum isolates Transactions of the Royal Society of Tropical Medicine and Hygien1996; 90: 181 – 182 .

72. Plowe CV , Djimde A, Bouare M, Doumbo O, Wellems TE.Pyrimethamine and proguanil resistance-conferring mutations in Plasmodium falciparum dihydrofolate reductase: polymerase chain reaction methods for surveillance in Africa . American Journal of Tropical Medicine and Hygiene 1995;52: 565 – 568 .

73. Mberu EK , Mosobo MK, Nzila AM, Kokwaro GO, Sibley CH, Watkins WM. The changing in vitro susceptibility pattern to pyrimethamine/sulfadoxine in Plasmodium falciparum field isolates from Kilifi, Kenya. American Journal of Tropical Medicine and Hygiene 2000; 62:396 – 401 .

74. Nzila AM , Mberu EK, Sulo J, et al . Towards an understanding of the mechanism of pyrimethamine-sulfadoxine resistance in Plasmodium falciparum : genotyping of dihydrofolate reductase and dihydropteroate synthase of Kenyan parasites . Antimicrobial Agents and Chemotherapy, 2000; 44: 991 – 996 .

75. Roper C, Pearce R, Bredenkamp B, et al. Antifolate anti-malarial resistance in southeast Africa: a population-based analysis. Lancet 2003 ;361(9364):1174-81.

76. Certain, L.K., Briceño M, Kiara SM, Nzila AM, Watkins WM, Sibley CH.Characteristics of Plasmodium falciparum dhfr haplotypes that confer pyrimethamine resistance Kilifi, Kenya, 1987–2006. Journal of Infectious Diseases 2008; 197: 1743–1751.

77. Amiga O, Djimdé AA, Hubert V, et al, Shared Asian origin of the triple-mutant dhfr allele in Plasmodium falciparum from sites across Africa. Journal of Infectious Diseases, 2007; 196: 165–172.

78. Anderson, TJ, Haubold B, Williams JT, et al. Microsatellite markers reveal a spectrum of population structures in the malaria parasite Plasmodium falciparum. Mol. Biol. Evol 2000;17: 1467–1482.

79. Peterson, D. Walliker and T.E. Wellems, Evidence that a point mutation in dihydrofolate reductase-thymidylate synthase confers resistance to pyrimethamine in falciparum malaria, Proceedings of the National Academy of Sciences , U.S.A., 1988; 85: 9114–9118.

80. Plowe, CV., Cortese JF,Djimde A,et al . Mutations in Plasmodium falciparum dihydrofolate reductase and dihydropteroate synthase and epidemiologic patterns of pyrimethamine- sulfadoxine use and resistance. Journal of Infectious Diseases 1997;176: 1590-1596.

81. Talisuna AO, Langi P, Mutabingwa TK, et al. Population-based validation of dihydrofolate reductase gene mutations for the prediction of sulfadoxine-pyrimethamine resistance in Uganda. Transactions of the Royal Society of Tropical

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Medicine and Hygiene 2003; 97: 338-342.

82. Noedl, H., Socheat, D. Satimai, W. Artemisinin resistant malaria in Asia. N. Engl. J. Med. 2009; 361:540–541

83. Dondorp AM, Nosten F, Yi P, Das D et al. Artemisinin resistance in Plasmodium falciparum malaria. N. Engl. J. Med 2009; 361: 455–467.

84. Dondorp AM, Yeung S, White L, Nguon C, et al. Artemisinin resistance: current statusand scenarios for containment. Nature Reviews Microbiology 2010 ; 8 : 272- 280.

85. Global plan for artemisinin resistance containment (GPARC). Accessed at: http://www.who.int/malaria/publications/atoz/artemisinin_resistance_containment_2011.pdf

86. Pongtavornpinyo W, Yeung S, Hastings IM, Dondorp AM, Day NP, White NJ. Spread of anti-malarial drug resistance: mathematical model with implications for ACT drug policies. Malar J 2008 ;7: 229.