GLOBAL REPORT ON ANTIMALARIAL DRUG EFFICACY AND DRUG RESISTANCE: 2000–2010

World Health Organization

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Abbreviations

ACT	artemisinin-based combination therapy
ARC3	artemisinin resistance project: pilot studies to confirm, characterize and plan for containment
DNA	deoxyribonucleic acid
HIV	human immunodeficiency virus
IC_{50}, IC_{90}, IC_{99}	50%, 90%, 99% inhibitory concentration
PCR	polymerase chain reaction
PfATPase6	gene encoding *P. falciparum* sarco-endoplasmic reticulum calcium ATPase 6
Pfcrt	gene encoding *P. falciparum* chloroquine resistance transporter
Pfdhfr	gene encoding *P. falciparum* dihydrofolate reductase
Pfdhps	gene encoding *P. falciparum* dihydropteroate synthase
Pfmdr1	gene encoding *P. falciparum* multidrug resistance 1 protein
Pfnhe-1	gene encoding *P. falciparum* Na^+/H^+ exchanger
Pfubp-1	gene encoding *P. falciparum* deubiquitinating enzyme
Pvdhfr	gene encoding *P. vivax* dihydrofolate reductase
Pvdhps	gene encoding *P. vivax* dihydropteroate synthase
Pvmdr1	gene encoding *P. vivax* multidrug resistance 1 protein
USA	United States of America
WHO	World Health Organization

Executive summary

BACKGROUND

Plasmodium resistance to antimalarial medicines is one of the major obstacles in the fight against malaria. Comprehensive, up-to-date understanding of the scope of antimalarial resistance is essential for protecting the recent advances in malaria control. Without regular monitoring and reporting of antimalarial drug resistance, the disease burden and the economic costs of malaria will rise dramatically. In addition, ineffective treatment resulting from drug resistance might lead more patients to rely on the unregulated private sector, increasing the risk of reliance on monotherapy, substandard and counterfeit medicines and subsequently leading to the emergence or further spread of drug resistance.

Measurement of drug resistance in malaria is complex. The tools that are used to monitor drug efficacy and evaluate drug resistance are described in this report. Studies of clinical and parasitological outcomes are the main sources of information on which national malaria control programmes base treatment policy; however, other studies are needed to confirm drug resistance. The aim of in vitro studies is to measure the intrinsic sensitivity of parasites to antimalarial drugs; molecular markers are used to identify genetic mutations related to antimalarial drug resistance in the parasite genome; and pharmacokinetic studies characterize antimalarial drug absorption, distribution, metabolism and elimination in the body. While each method makes a contribution to a more complete understanding of antimalarial drug resistance, therapeutic efficacy studies remain the gold standard for guiding drug policy. This report is therefore based primarily on the results of therapeutic efficacy studies.

WHO GLOBAL DATABASE ON ANTIMALARIAL DRUG EFFICACY

The results of therapeutic efficacy studies conducted within national malaria control programmes and in research institutes in which antimalarial treatment efficacy was diligently monitored, were systematically collected and analysed for the WHO global database on antimalarial drug efficacy. As of June 2010, the database contained the clinical and parasitological outcomes of 3932 studies conducted between 1996 and June 2010 on *P. falciparum* malaria; however, only those studies conducted during the past 10 years and which met the inclusion criteria were included in this report. The analysis was thus based on 1120 studies representing 81 848 patients. The results are presented for monotherapy and for combination therapy, by country and by drug. Published findings and country data on the efficacy of *P. vivax* malaria treatment were also reviewed, but a similar database does not yet exist for *P. vivax* malaria.

WHO has recommended artemisinin[1]-based combination therapies (ACTs) as first-line treatment for uncomplicated *P. falciparum* malaria since 2001, and, during the past decade, most malaria-endemic countries shifted their national treatment policies to ACTs. Nonetheless, the efficacy studies of monotherapies are of interest because some of these monotherapies are components of ACTs; as the efficacy of a monotherapy is often correlated with that of the combinations in which it occurs, the results of these studies can provide an indication of the overall efficacy of ACTs. Chloroquine is still being used as first-line treatment in some countries and studies of oral artesunate monotherapy are conducted to improve understanding of the spread and mechanism of artemisinin resistance.

[1] Unless otherwise indicated, the word 'artemisinin' is used in this document to refer to artemisinin and its derivatives, artesunate, artemether and dihydroartemisinin.

The main findings from the analysis of the WHO global database on antimalarial drug efficacy are:

- Artemether–lumefantrine remains highly effective in most parts of the world, with the exception of Cambodia. More studies are needed to determine the current efficacy of artemether–lumefantrine in Africa, as more than 85% of the studies in the database were completed in 2007 or earlier.
- At least one study with a high treatment failure rate (≥ 10%) was reported from six of the 23 African countries that have adopted artesunate–amodiaquine. A high treatment failure rate for this combination was also observed in four studies in Indonesia.
- The efficacy of artesunate–mefloquine is lowest in areas where mefloquine resistance is prevalent, for example in the Greater Mekong subregion. In Africa and the Americas, the combination remains highly effective.
- The failure rates of artesunate–sulfadoxine–pyrimethamine are high in regions where resistance to sulfadoxine–pyrimethamine is high. This ACT remains effective in countries in which the combination is used as first-line treatment.
- Little information was available on the therapeutic efficacy of dihydroartemisinin–piperaquine. Studies conducted in some parts of Africa and in the Greater Mekong subregion indicate high efficacy. More studies are needed to confirm its current efficacy in endemic countries.

Chloroquine remains the treatment of choice for *P. vivax* malaria in areas where it remains effective. Treatment failure on or before day 28 or prophylactic failure has been observed in Afghanistan, Brazil, Cambodia, Colombia, Guyana, Ethiopia, India, Indonesia, Madagascar, Malaysia (Borneo), Myanmar, Pakistan, Papua New Guinea, Peru, the Republic of Korea, Solomon Islands, Thailand, Turkey, Sri Lanka, Vanuatu and Viet Nam. Confirmation of true chloroquine resistance, however, requires additional studies of drug concentrations achieved in blood. The spread of chloroquine-resistant *P. vivax* is therefore not entirely clear. At least one confirmed case of chloroquine-resistant vivax malaria was reported in Brazil, Ethiopia, Indonesia, Malaysia (Borneo), Myanmar, Solomon Islands, Thailand, Papua New Guinea and Peru. ACTs are now recommended for the treatment of chloroquine-resistant *P. vivax,* particularly where ACTs have been adopted as first-line treatment for *P. falciparum* malaria.

ARTEMISININ RESISTANCE AND CONTAINMENT

The critical role of monitoring for drug efficacy was demonstrated on the Cambodia–Thailand border area, where *P. falciparum* resistance to artemisinin has emerged. Therapeutic efficacy studies conducted by the national malaria control programmes of Cambodia and Thailand were the first to show an increase in the proportion of patients who were still parasitaemic on day 3, which indicates a change in the pattern of parasite susceptibility to artemisinins and is probably the first stage of artemisinin resistance. An increase in the proportion of patients who were still parasitaemic on day 3 was also reported on the Myanmar–Thailand and China–Myanmar borders and in one province of Viet Nam; the situation in these other sites is less severe than on the Cambodia–Thailand border but merits careful monitoring and early response.

Despite the changes observed in parasite sensitivity to artemisinins, the clinical and parasitological efficacy of ACTs is not yet compromised, even in the Greater Mekong subregion. Nonetheless, the efficacy of both components of the combination is at risk. One of the main determinants of clinical and parasitological failure after treatment with an ACT is the local efficacy of the partner drug; use of an ACT with an ineffective partner medicine could increase the risk for the development or spread of artemisinin resistance. Similarly, if the efficacy of the artemisinin component is lost, the efficacy of the partner drug could be jeopardized.

Immediately after confirmation of artemisinin resistance on the Cambodia–Thailand border, WHO, in collaboration with national malaria control programmes and other partners, initiated a containment project with the goal of stopping the spread of artemisinin-resistant parasites by removing selection pressure and by ultimately eliminating *P. falciparum*-resistant parasites from the area. In view of the emergence of

artemisinin resistance in the Greater Mekong subregion and the threat of its spread to other areas, malaria control and elimination efforts must be intensified. For this purpose, the WHO Global Malaria Programme is launching the *Global plan for artemisinin resistance containment*, with the overarching goal of protecting ACTs as an effective treatment for *P. falciparum* malaria. Global and local partners will be encouraged to become involved in containing and ultimately eliminating artemisinin resistance if and where it emerges, while simultaneously preventing its spread to new locations. Loss of artemisinin to resistance, like all preceding antimalarials, would represent a reversal of the global achievements in malaria control.

RECOMMENDATIONS

WHO emphasizes the need for countries to conduct therapeutic efficacy studies of the antimalarial medicines being used as first- and second-line treatment as the information derived facilitates early detection and subsequent prevention of the spread of drug resistance. Standardized methods allow comparison of data across geographical regions and over time. Monitoring is essential for timely changes to treatment policy, which should be initiated when the treatment failure rate exceeds 10% at the end of follow-up (28 or 42 days, depending on the half-life of the medicines). Monitoring of ACTs should also include evaluation of the proportion of patients who still have parasites on day 3, in order to detect what is considered to be the first sign of artemisinin resistance.

Countries are requested to monitor the efficacy of chloroquine and ACTs against *P. vivax*, either at existing sentinel sites being used to monitor their therapeutic efficacy against falciparum malaria or at new sites in areas where vivax malaria is prevalent. In 2011, WHO will establish a database on *P. vivax*, which will facilitate understanding of the trends and the extent of *P. vivax* resistance to chloroquine. The database will also help to determine which ACTs are the most appropriate for treating vivax malaria.

The *Global plan for containment of artemisinin resistance* should be implemented immediately by all those involved. The present time may be a limited window of opportunity for containing or eliminating artemisinin resistance where it exists, before it spreads to high-transmission areas, endangering all recent advances in malaria control. The urgency is all the greater because no other antimalarials are available that offer the same efficacy and tolerability as ACTs, and few alternatives exist for the immediate future.

Introduction

Early, effective treatment of malaria is the cornerstone of malaria control, and appropriate selection of first- and second-line antimalarial medicines for country programmes is based entirely on the efficacy of the medicines against the malaria parasite. Monitoring the therapeutic efficacy of antimalarial medicines is therefore a fundamental component of treatment strategies. As the parasite evolves continuously to develop resistance to medicines, continuous global monitoring and reporting of drug efficacy and parasite resistance are needed. Only a programme of surveillance of therapeutic efficacy and antimalarial drug resistance will allow detection of changing patterns of parasite susceptibility and timely revision of national (and global) malaria treatment policies.

Chapter 1 distinguishes between antimalarial drug efficacy and resistance and describes the mechanisms by which antimalarial drug resistance emerges and spreads.

Chapter 2 outlines the scientific methods used to monitor drug efficacy and to detect and confirm drug resistance. The strengths and weaknesses of each method are described, indicating the need for further refinement and standardization.

In Chapter 3, the results of studies of therapeutic efficacy and drug resistance are given for all antimalarial treatments for which data were available, over time and by geographical subregion. The global picture is based mainly on the therapeutic efficacy studies ('in vivo tests') in the database maintained by the WHO Global Malaria Programme, complemented by published studies with in vitro assays, molecular markers and measurements of antimalarial drug concentrations. All the available information on drug efficacy reported to WHO and published in the literature are compiled in the database, representing the largest collection of studies on antimalarial drug efficacy that have been reviewed and standardized for analysis. After the emergence of chloroquine resistance, WHO supported drug resistance monitoring by regularly updating the standardized therapeutic efficacy study protocol, supporting and coordinating the collection, analysis and reporting of data and promoting appropriate use of the additional tests needed to confirm resistance. The original research included in the analysis was conducted in several hundred research institutes and national malaria control programmes, which are either currently monitoring drug efficacy and drug resistance or conducted related studies in the past.

Chapter 4 describes the recent emergence of resistance to artemisinin on the Cambodia–Thailand border and coordination of the containment response in the Greater Mekong subregion. Reduced parasite susceptibility to artemisinins was first suspected during routine surveillance of therapeutic efficacy by the national malaria control programmes of Cambodia and Thailand. If there had not been regular monitoring, the subtle changes in parasite sensitivity would not have been detected. Early detection enabled scientists to make more detailed investigations to confirm the presence of resistance, characterize the molecular changes in the parasite, confirm that these changes were associated with true resistance and plan for containment. In contrast to the situation with other antimalarial medicines to which *P. falciparum* eventually became fully resistant, in this situation national malaria control programmes and the international malaria community had an opportunity to respond to early signs of resistance with an intensive containment effort. The global response is particularly critical given that no other drugs are currently available to replace artemisinins should they cease to be effective. The information in this report and early experience with regional and country-level containment activities illustrate the urgent, unprecedented need for a well-coordinated global response to the threat of artemisinin resistance. A detailed plan for this global response, the *Global plan for artemisinin resistance containment*, will be released in early 2011 by WHO.

1. Antimalarial drug efficacy and drug resistance

This section provides a review of antimalarial drug efficacy and resistance, including the mechanisms by which resistance emerges and spreads, and an assessment of the public health consequences of drug resistance.

1.1 Definitions

DRUG RESISTANCE

For decades, drug resistance has been one of the main obstacles in the fight against malaria. To date, drug resistance has been documented in three of the five malaria species known to affect humans in nature: *P. falciparum*, *P. vivax* and *P. malariae*. In 1967, WHO defined 'drug resistance' as the ability of a parasite strain to survive or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within the tolerance of the subject (WHO, 1967). This definition was later modified to include the sentence: "The form of the drug active against the parasite must be able to gain access to the parasite or the infected erythrocyte for the duration of the time necessary for its normal action" (Bruce-Chwatt et al., 1986). This addition took into account a new development in the understanding of human metabolism of sulfonamide. As the pharmacokinetics of antimalarial medicines varies widely among individuals, the definition of resistance is enhanced if the concentration profile of the active drug concerned is also taken into consideration. For example, in the case of a prodrug, which is not active in the ingested form and requires chemical conversion by metabolic processes to become pharmacologically active, the definition should also include a requirement for a 'normal' profile of the biologically active metabolite. Because of the particular mode of action of artemisinins, the definition of resistance may require further discussion and clarification (see section 4.4).

Drug resistance is complicated by cross-resistance, which can occur among drugs that belong to the same chemical family or which have similar modes of action (Box 1). Multidrug resistance of *P. falciparum* is seen when the parasite is resistant to more than two operational antimalarial compounds of different chemical classes and modes of action. Generally, the two classes first affected are the 4-aminoquinolines and the antifolates (diaminopyrimidine, sulfonamides). Drug resistance results in a delay in or failure to clear asexual parasites from the blood, which allows production of the gametocytes that are responsible for transmission of the resistant genotype.

The rise of antimalarial drug resistance has changed the global epidemiology of malaria. Box 2 lists the implications for malaria control.

TREATMENT FAILURE

Treatment failure is defined as an inability to clear malarial parasitaemia or resolve clinical symptoms despite administration of an antimalarial medicine. Treatment failure is not, however, always due to drug resistance, and many factors can contribute, mainly by reducing drug concentrations. These factors include incorrect dosage, poor patient compliance in respect of either dose or duration of treatment, poor drug quality and drug interactions. Even after supervised administration of a full regimen of an antimalarial medicine, individual variations in pharmacokinetics might also lead to treatment failure because of poor absorption, rapid elimination (e.g. diarrhoea or vomiting) or poor biotransformation of prodrugs.

Only by studying therapeutic efficacy (to estimate the treatment failure rate) can the influence of some of these factors on treatment outcome be ruled out. Therapeutic efficacy studies are conducted in a controlled environment, in which drug administration is supervised, the results of microscopic examinations of blood films are validated, and the origin and quality of the drugs are verified. The outcome of the study is influenced by a combination of a human factor (immunity), a parasite factor (drug resistance) and individual variation leading to differences in the availability of the drug (pharmacokinetics) (Rogerson, Wijesinghe & Meshnick, 2010). For example, an adult living in an area of high transmission might be able to eliminate resistant parasites even if the medicine is not fully effective, because of acquired immunity (Djimdé et al., 2003). Conversely, a non-immune child infected with drug-sensitive parasites who has severe gastrointestinal problems may experience therapeutic failure because of poor absorption (Herzog et al., 1982).

While therapeutic efficacy studies can help to predict the likelihood of drug resistance, additional tools are needed to confirm antimalarial drug resistance. First, it must be proven that the parasites are recrudescent in a patient who recently received treatment. The parasites are genotyped to distinguish between those that are recrudescent and those that caused a new infection. Evidence must be obtained that the patient had an adequate blood concentration of the drug or its metabolites, typically for at least four parasitic cycles (White, 1998). This can be confirmed by pharmacokinetic analyses of blood samples. Confirmation of resistance to antimalarial medicines with a short half-life (e.g. quinine and artemisinins) is more complex and is discussed later.

Methods such as in vitro tests and molecular analysis, if available and validated, and measurements of drug concentrations complement the results of therapeutic efficacy studies and can be important for monitoring. The results of these additional tests must, however, be interpreted with caution, as they do not always correlate well with the results of therapeutic efficacy studies, and the predictive usefulness of some of these tests remains to be defined (Basco, 2007; Picot et al., 2009).

BOX 1. PRINCIPLE AVAILABLE ANTIMALARIAL DRUGS

Chemical family	Drugs
4-Aminoquinolines	Chloroquine, amodiaquine, piperaquine
Amino-alcohols	Quinine, quinidine, mefloquine, halofantrine, lumefantrine
Sulfonamides and sulfones	Sulfadoxine, sulfalene, dapsone
Biguanides	Proguanil, chlorproguanil
Diaminopyrimidine	Pyrimethamine
8-Aminoquinoline	Primaquine
Sesquiterpene lactones	Artemisinin, arteether, artemether, artesunate, dihydroartemisinin
Naphthoquinone	Atovaquone
Antibiotics	Azythromycin, clindamycin, doxycycline, tetracycline

BOX 2. EFFECTS OF ANTIMALARIAL DRUG RESISTANCE ON GLOBAL MALARIA CONTROL	
Disease burden	• The appearance of chloroquine resistance in Africa led to an increase in hospital admissions (Zucker et al., 1996). • Increasing mortality trends were found at community level (Trape et al., 1998; Korenromp et al., 2003). • Ineffective treatment causes anaemia and low birth weight (Björkman, 2002) and renders the health of children and adults infected with *P. falciparum* or *P. vivax* more fragile (Tjitra et al., 2008). • Resistance to antimalarial drugs was implicated, at least partially, in malaria epidemics (Warsame et al., 1990). • Resistance to antimalarial drugs is associated with increased transmission (Price & Nosten, 2001).
Economic cost	• Resistance to antimalarial drugs has increased the global cost of controlling the disease, including the cost of new drug development (Phillips & Phillips-Howard, 1996). • Therapeutic failure requires consultation at a health facility for further diagnosis and treatment, resulting in loss of working days for adults, absence from school for children and increased cost to the health system (Talisuna, Bloland & D'Alessandro, 2004).
Changes to distribution of malaria species	• The proportion of *P. falciparum* malaria has changed, such as an increase with respect to *P. vivax* (Dash et al., 2008).
Access to high-quality treatment	• Ineffective treatment in the public sector due to resistance could lead to greater reliance of patients on the unregulated private sector, which in turn could increase the use of monotherapies or substandard and counterfeit medicines and increase the risk for drug resistance.

1.2 Emergence and spread of resistance to antimalarial drugs

EMERGENCE OF GENETIC MUTATIONS

The development of resistance can be considered to occur in two phases. In the first phase, an initial genetic event produces a resistant mutant (de novo mutation); the new genetic trait gives the parasite a survival advantage against the drug. In the second phase, the resistant parasites are selected for and begin to multiply, eventually resulting in a parasite population that is no longer susceptible to treatment.

Genetic events that confer antimalarial drug resistance are spontaneous and rare. They are considered to occur randomly, independently of the drug. These events are characterized by gene mutations or changes in the number of copies of genes that determine the drug's target or that affect pumps that regulate the intraparasitic concentrations of the drug. A single genetic event may be all that is required; in other cases, multiple independent events may be necessary (Valderramos et al., 2010a). For example, in the case of de novo resistance to chloroquine, molecular epidemiological studies have suggested that resistant mutants emerged independently at a limited number of sites. In Africa, the advent of chloroquine resistance was not

linked to the appearance of a new mutation there but to the slow, gradual spread of chloroquine-resistant parasites from South-East Asia, which finally arrived in East Africa in 1978 (Sá et al., 2009). In contrast, resistance to antifolate and atovaquone arises more frequently and is easily induced in experimental models (Peters, 1969; Looareesuwan et al., 1996; Pearce et al., 2009; Vinayak et al., 2010a).

One study suggested that *P. falciparum* in South-East Asia has an inherent propensity to develop drug resistance through genetic mutation (Rathod, McErlean & Lee, 1997). It was shown in microsatellite marker studies that *P. falciparum* resistant to chloroquine or highly resistant to pyrimethamine both originated in South-East Asia and subsequently spread to Africa (Wootton et al., 2002; Roper et al., 2004). The emergence of resistance to mefloquine arose rapidly on the western border of Cambodia and on the north-west border of Thailand in the 1980s (Wongsrichanalai et al., 2001). Epidemiological studies have since shown that the molecular change that led to mefloquine resistance occurred in multiple, independent events, suggesting that it arose in several different places (Vinayak et al., 2010b).

Despite the random nature of the genetic emergence of drug resistance, some measures can be taken to mitigate the risk. For example, non-immune patients infected with large numbers of parasites who receive inadequate treatment (because of e.g. poor drug quality, poor adherence, vomiting of an oral treatment) are a potent source of de novo resistance. This underscores the need for correct prescribing, adherence to prescribed drug regimens and provision of effective treatment regimens, especially for hyperparasitaemic patients (White et al., 2009).

SPREAD OF ANTIMALARIAL DRUG RESISTANCE

Further selection of drug-resistant mutants probably occurs when parasites are exposed to subtherapeutic drug concentrations (White & Pongtavornpinyo, 2003). An inadequate drug concentration will eradicate only those parasites that are still sensitive. In experimental models (i.e. in vitro, animals or volunteers), drug-resistant mutations have even been selected without mosquito passage (i.e. without meiotic recombination) when large numbers of parasites were exposed to subtherapeutic drug concentrations (Peters, 1987). The resistant parasite population that remains can subsequently spread to other patients during malaria transmission.

Once drug-resistant parasites have emerged and are selected over sensitive ones, it is difficult to prevent the spread of drug resistance. The removal of drug pressure may reduce the chance that resistant mutants will survive, as the 'fitness cost' of the resistance mechanism is likely to diminish the prevalence of resistance (Felger & Beck, 2008). Drug pressure cannot, however, always be removed. Further, the propagation of newly emerged resistance depends on the recrudescence and subsequent transmission of an infection that has generated a de novo resistant malaria parasite (White, 1999). Therefore, gametocyte production from the recrudescent resistant infection must be prevented by administration of early, appropriate treatment.

There is a time window for selection, during which drug levels are such that the resistant parasite strain has a survival benefit over sensitive ones. Before this time, the drug concentrations are high enough to kill the partly resistant parasites; after this time, the drug concentrations are too low, and the sensitive parasite strains will survive (Stepniewska & White, 2008). The subsequent spread of resistant mutant malaria parasites is facilitated by administration of drugs with long elimination phases. The residual antimalarial activity that is present during the post-treatment period serves as a 'selective filter', which prevents infection by sensitive parasites but allows infection by resistant parasites. Drugs such as chloroquine, mefloquine and piperaquine, which persist in the blood for months, provide a selective filter long after their administration has ceased (Yeung et al., 2004).

FACTORS THAT INFLUENCE THE EMERGENCE AND SPREAD OF RESISTANCE

Several factors influence the emergence and spread of drug-resistant parasites. A list of these factors is given in Box 3 (White, 1999; White & Pongtavornpinyo, 2003).

BOX 3. FACTORS THAT INFLUENCE THE DEVELOPMENT OF ANTIMALARIAL DRUG RESISTANCE

- the intrinsic frequency with which the genetic changes occur;
- the degree of resistance conferred by the genetic change;
- the 'fitness cost' of the resistance mechanism;
- the proportion of all transmissible infectious agents exposed to the drug (selection pressure);
- the number of parasites exposed to the drug;
- the concentrations of drug to which the parasites are exposed;
- the pharmacokinetics and pharmacodynamics of the antimalarial medicine;
- individual (dosing, duration, adherence) and community (quality, availability, distribution) patterns of drug use;
- the immunity profile of the community and the individual;
- the simultaneous presence of other antimalarial drugs or substances in the blood to which the parasite is not resistant; and
- the transmission intensity.

Despite ongoing debate about whether resistance arises more rapidly in low- or high-transmission settings (Hastings & Mackinnon, 1998; Mackinnon & Hastings, 1998), epidemiological studies have implicated low-transmission settings as the primary origin of drug resistance (Roper et al., 2004). This is probably due to the fact that in low-transmission areas, most malaria infections are symptomatic, and therefore proportionally more people receive treatment, providing more opportunities for selection.

High-transmission areas appear to be less susceptible to the emergence of drug resistance, primarily because most malaria infections are asymptomatic and infections are acquired repeatedly throughout life. In high-transmission areas, malaria-experienced individuals gradually acquire partial immunity ('premunition'), and the infection is controlled, usually at levels below those that cause symptoms. The rate at which premunition is acquired depends on the intensity of transmission. Furthermore, complex polyclonal infections in semi-immune people allow possible outbreeding of multigenic resistance mechanisms or competition in the host or the mosquito between less-fit resistant strains and more-fit sensitive strains (Dye & Williams, 1997).

Immunity can also considerably reduce the emergence and spread of resistance. Host defense has a major antiparasitic effect, and any spontaneously generated drug-resistant mutant malaria parasite must contend not only with the antimalarial drug concentrations but also with host immunity, which kills parasites regardless of their antimalarial resistance and reduces the probability of parasite survival (independently of drugs) at all stages of the transmission cycle. Immunity acts by non-selectively eliminating blood-stage parasites, including the rare de novo resistant mutants, and also improves cure rates, even with failing drugs, thereby reducing the relative transmission advantage of resistant parasites. Even if a resistant mutant survives the initial drug treatment and multiplies, the likelihood that this will result in sufficient gametocytes for transmission is reduced as a result of immunity to the asexual stage (which reduces the multiplication rate and lowers the density at which the infection is controlled) and to the sexual stage.

2. Monitoring antimalarial drug efficacy and drug resistance

Four main methods are used to monitor antimalarial drug efficacy and drug resistance (therapeutic efficay studies, in vitro tests, use of molecular markers and measurement of drug concentrations). Therapeutic efficacy studies allow measurement of the clinical and parasitological efficacy of medicines and the detection of subtle changes in treatment outcome when monitored consistently over time. They are considered the gold standard for determining antimalarial drug efficacy, and their results are the primary data used by national malaria control programmes to make treatment policy decisions. While therapeutic efficacy studies conducted according to a standard protocol provide an excellent indication of drug efficacy, additional studies are needed to confirm and characterize drug resistance and may be of some use in surveillance. These studies include in vitro studies of changes in the parasite phenotype, molecular marker studies of genetic mutations of the parasite and pharmacokinetic analyses of drug concentrations in blood. These four methods are described in detail below and summarized, with their advantages and disadvantages, in Table 1.

2.1 Therapeutic efficacy studies

HISTORY OF THE DEVELOPMENT OF A STANDARDIZED THERAPEUTIC EFFICACY PROTOCOL

After the emergence of chloroquine resistance, malaria experts agreed that a standard test was needed that could be used in all national malaria control programmes to evaluate the in vivo response of *P. falciparum* to chloroquine. The first standard protocol for monitoring antimalarial drug efficacy was formulated by a WHO scientific group in 1964 (WHO, 1965) and was updated in 1967 (WHO, 1967) and 1972 (WHO, 1973). Consistent use of the standardized WHO protocol for monitoring antimalarial drug efficacy has become essential for comparing results within and between countries over time. The protocol was revised in 1996, 2001 and 2009, in order to keep it relevant for the changing patterns of drug-resistant malaria and for the needs of the national programmes that were monitoring drug efficacy. The revisions also incorporated recommendations for the use of new techniques for the detection and confirmation of drug-resistant parasites and the new medicines available for treatment. A summary of each protocol revision is given in Table 2. This section describes the protocols used between 1973 and 2009 and the rationale for each revision.

The 1973 WHO protocol required adherence to set criteria for administration of a standard treatment regimen and regular examination of blood for a fixed period, e.g. 7 or 28 days for chloroquine. The protocol had several limitations. First, as it was originally prepared for chloroquine, it focused on parasitological rather than clinical outcomes. Secondly, it required daily blood sampling during the first week and weekly tests thereafter if follow-up extended beyond 7 days. Frequent blood tests were burdensome for both the patient and the clinician. Although the protocol did not recommend the inclusion of asymptomatic carriers with low parasitaemia, studies were subsequently performed in Africa with asymptomatic semi-immune schoolchildren. This led to inadequate interpretation of the efficacy of chloroquine. Finally, subsequent attempts to simplify the 1973 WHO in vivo test were unsatisfactory. The short surveillance period of 7 days was found to result in an underestimate of the true percentage of therapeutic failures, especially for drugs with a long half-life. As only two studies demonstrated that the presence of parasites on day 2 or 3 was predictive of treatment failure (ter Kuile et al., 1995; Bloland et al., 1998), short tests were not widely endorsed.

TABLE 1. Methods for monitoring antimalarial drug efficacy and drug resistance

	THERAPEUTIC EFFICACY STUDY	IN VITRO SENSITIVITY ASSAY	MOLECULAR MARKERS	DRUG CONCENTRATION MEASUREMENT
Definition	Treatment of symptomatic patients infected only with *P. falciparum* with a standard dose of an antimalarial drug and subsequent follow-up of parasitaemia and clinical signs and symptoms over a defined period (response of the host–parasite system to the drug)	Cultivation of *P. falciparum* parasites in vitro with a range of antimalarial drug concentrations (response of the parasites to the drug)	Detection of genetic markers that modify drug target (enzymes) or drug transporter functions or affinities	Antimalarial drug and/or active metabolite(s) in whole blood, plasma or serum
Indications	Gold standard for monitoring antimalarial drug efficacy and for guiding drug policy	Reduced parasite response to antimalarial drug Early warning system (adjunct to therapeutic efficacy study)	Resistance-related mutations or amplification Early warning system (adjunct to therapeutic efficacy study)	Used to distinguish between treatment failures due to antimalarial drug resistance and suboptimal drug exposure, to interpret adverse effects, to identify subgroups who need drug adjustment
Advantages	Simple method with minimal training required (except microscopy) Minimal equipment and supplies required	Avoids host confounding factors Accurate for detecting true drug resistance Provides quantitative results Multiple tests can be performed with a single isolate, and several drugs can be assessed simultaneously Experimental drugs can be tested (except prodrugs) In vitro resistance precedes in vivo resistance	Avoids host confounding factors Accurate for detecting true drug resistance Samples on filter paper easily obtained, transported and stored Multiple tests can be performed with a single filter paper, and molecular targets of several drugs can be characterized If known, targets of new and experimental drugs can be tested Mutations may precede frank in vivo resistance Allows population-level screening, including prevalence of potentially resistant parasites in asymptomatic individuals or infected mosquitoes	Facilitates accurate definition of true drug resistance For slowly eliminated drugs, day 7 concentrations are a more feasible yet reliable measure of the parasites' exposure to drug than repeated sampling required for area under the curve* Multiple tests can be performed with a single blood sample, and several drugs can be assayed simultaneously Samples on filter paper easily obtained, transported and stored Provides quantitative results Potentially possible on non-invasive urine or saliva specimens
Drawbacks	Interference of immunity, previous drug intake, variation of drug absorption or metabolism Misclassification of reinfection and recrudescence Treatment failures do not necessarily reflect the level of true drug resistance Difficult to conduct in areas of low transmission, given the limited numbers of eligible patients Overestimation of early treatment failures for slowly acting drugs Numerous local adaptations and modifications result in poor comparisons between sites Long duration of patient monitoring may result in high patient loss to follow-up	Correlation with therapeutic efficacy study not fully established Presence of mixed population with different drug sensitivity phenotypes Expensive equipment and supplies required Training required Numerous methods available that are not always comparable Lack of standardized in vitro protocol Threshold of resistance not validated In vitro adaptation may stress parasites in ways that differ from population selection in vivo	Correlation with therapeutic efficacy study results not fully established Presence of mixed population with mixed alleles Expensive equipment and supplies required Training required Identified for a limited number of antimalarial drugs Lack of standardized protocol including sample collection and DNA extraction	Expensive equipment and supplies required Training required Filter paper sampling not possible for all antimalarial drugs (in particular artemisinins) and may require venous blood samples Lack of standardized methods Interpretation requires accurate dosing history and record of timing of sample collection

* Area under the concentration–time curve

TABLE 2. Evolution of the WHO protocol for monitoring antimalarial drug efficacy (1964–2009)

	FOLLOW-UP (DAYS)	OUTCOME CLASSIFICATION	LIMITATIONS	MODIFICATIONS	CONSENSUS MEETINGS	REASON FOR PROTOCOL DEVELOPMENT / MODIFICATION
1964–1972	7 or 28	Parasitological outcomes only: S, RIII, RII, RI	Daily blood sampling for first 7 days burdensome for clinicians and patients No consideration of clinical response Inclusion and exclusion criteria not clearly defined	Not applicable	WHO scientific groups, (WHO, 1965; WHO, 1967; WHO, 1973)	Emergence of resistance to chloroquine
1996	14	ACR, ETF, LCF	Protocol limited to high-transmission areas	More emphasis on clinical symptoms and outcome focused on 'clinical cure'	Intercountry workshop in Mangochi, Malawi, August 1996 (WHO, 1996)	Simplification needed Need protocol targeting groups at risk in high-transmission areas
2001	14 or 28	ACPR, ETF, LCF, LPF	Protocol did not consider clinical and parasitological cure to be the objective in high-transmission areas	Two separate protocols were created to accommodate the differences between high- and low-to-moderate-transmission areas: in high-transmission areas, there was an emphasis on clinical cure, although LPF was still included as a possible treatment outcome, whereas in low-to-moderate-transmission areas, there was an emphasis on both clinical and parasitological cure	Manila, Philippines, October 1996 Manaus, Brazil, March 1998 (WHO, 1998) Phnom Penh, Cambodia, October 2000 Geneva, Switzerland, December 2001 (WHO, 2003)	Need protocol for low-transmission areas and areas prone to epidemics Need a longer follow-up period
2009	28 or 42	ACPR, ETF, LCF, LPF		Same definitions of treatment response at all levels of malaria transmission Administration of rescue treatment to patients with parasitological treatment failure at all levels of malaria transmission 28 or 42 days' follow-up, depending on medicine Systematic use of PCR	Technical expert group meetings on guidelines for treatment of malaria (WHO, 2006; WHO, 2010)	Harmonization of the two separate protocols Incorporation of PCR technology to distinguish between reinfection and recrudescence Response to outcomes of technical expert group

ACPR, adequate clinical and parasitological response; ACR, adequate clinical response; ETF, early treatment failure; LCF, late clinical failure; LPF, late parasitological failure; PCR, polymerase chain reaction. For definitions of RI, RII, RIII and S, see Box 9 in Annex 1.

In 1996, a revised standard protocol was developed by the Centers for Disease Control and Prevention, Atlanta GA, United States of America (USA) and WHO, which was designed to assess the therapeutic efficacy of antimalarial drugs for *P. falciparum* infections in febrile infants and young children living in high-transmission areas (WHO, 1996).

Nevertheless, a protocol with clinical and parasitological outcomes was still needed for areas of low transmission and for areas with large cyclical variations in malaria, sometimes verging on epidemics, which included most malaria-endemic regions outside Africa. After several interregional meetings held between 1996 and 2000, a modified protocol was adopted by experts in 2001 (WHO, 2003). The 2001 WHO protocol was the first to make a clear distinction between patients in high- and low-to-moderate-transmission areas, mainly with regard to inclusion criteria and the management of asymptomatic parasitological failures. Specifically, the revised protocol emphasized both clinical and parasitogical cure for patients in low-to-moderate-transmission areas only. These patients were considered at greater risk for treatment failure after parasitological failure, due to lower immunity; however, this distinction was revised in the 2009 WHO protocol.

Further protocol revisions were made after technical expert group meetings to discuss guidelines for the treatment of malaria in 2005 and 2008. These meetings resulted in a consensus on clinical and parasitogical cure of the disease for patients in all transmission areas, as effective treatment prevents the development of severe malaria and reduces morbidity and mortality associated with treatment failure. From a clinical point of view, all parasites must be eliminated in order to avert the risk for anaemia and recurrent clinical signs and symptoms. From a public health perspective, clearance of infection also reduces gametocyte carriage, and thus transmission, and prevents resistance. In response to the expert group recommendations, the following modifications were made to the most recent version of the WHO standard protocol (WHO, 2009a):

- the same definitions of treatment responses applied at all levels of malaria transmission, with slight adjustment of patient inclusion criteria (see below);
- rescue treatment to be administered to patients with parasitological treatment failure at all levels of malaria transmission;
- standard follow-up of 28 or 42 days, the latter for medicines with longer elimination half-lives; and
- genotyping by polymerase chain reaction (PCR) to distinguish between recrudescence and reinfection.

THERAPEUTIC EFFICACY STUDY PROTOCOL 2009

Detailed descriptions of the inclusion and exclusion criteria, calculation of sample size, length of follow-up, assessment criteria, data analysis and management, ethical considerations and quality control are available in *Methods for surveillance of antimalarial drug efficacy* (WHO, 2009a). In brief, the latest version of the therapeutic efficacy study protocol includes an evaluation of the first- and second-line drug combinations that are usually administered over 3 days. The study is a one-armed prospective study of clinical and parasitological responses after administration of antimalarial treatment to children aged 6–59 months. This age group is preferred because of their low immunity, which is less likely to influence the treatment outcome. In areas of low-to-moderate transmission, where it is difficult or time-consuming to enrol only children under 5 years of age, children over 5 years and adults can be included, although this might result in an underestimate of the true level of drug resistance, as adults tend to respond better to treatment than children. In order to avoid inclusion of asymptomatic carriers, only patients with a minimum of 2000 parasites per µl (or 1000 parasites per µl in areas of low-to-moderate transmission) are included. Treatment outcomes are classified as early treatment failure, late clinical failure, late parasitological failure or adequate clinical and parasitological response. Patients who are lost to follow-up or excluded (because

of e.g. self-medication, development of concomitant febrile infections, refusal to continue participation) are removed or censored from the analysis.

A 28-day follow-up is adequate to detect most cases of late treatment failure for drugs with elimination half-lives of less than 7 days (i.e. amodiaquine, artemisinins, atovaquone–proguanil, chloroquine, halofantrine, lumefantrine, quinine and sulfadoxine–pyrimethamine). For medicines with longer elimination half-lives (i.e. mefloquine, piperaquine), a follow-up of at least 42 days is needed (Stepniewska et al., 2004). As most failures in clinical studies of ACTs occur after day 21, the minimum duration of follow-up should be 28 days, regardless of the level of transmission. Studies should be complemented by molecular tests in order to distinguish cases of recrudescence from reinfection (WHO, 2008a).

THERAPEUTIC EFFICACY STUDIES AND NATIONAL MALARIA TREATMENT POLICY

Therapeutic efficacy studies, when conducted regularly according to a standard protocol, allow national malaria control programmes to maintain relevant, effective national malaria treatment policies. With a few exceptions, all national malaria policy changes have been based solely on the results of therapeutic efficacy studies. In other cases, the decision to change policy has been based on therapeutic efficacy studies and additional confirmatory evidence from in vitro and molecular marker studies. For example, in South Africa, data on drug sensitivity in vitro were considered when chloroquine was abandoned as a first-line drug. In Mali, the results of molecular marker studies were fundamental to the decision to use sulfadoxine–pyrimethamine instead of chloroquine during an epidemic outbreak (Djimdé et al., 2004). In the United Republic of Tanzania, geographical monitoring of the molecular markers of sulfadoxine–pyrimethamine provided the additional evidence needed to help policy-makers change the national policy to ACTs (Schönfeld et al., 2007). Nevertheless, the results of therapeutic efficacy studies remain the primary reference for ministries of health in updating treatment strategies and policies. The efficacy of national first- and second-line antimalarial treatments should be monitored at least once every 2 years, as recommended in the WHO standard protocol for monitoring drug efficacy. A change in an antimalarial medicine recommended in the national malaria treatment policy should be initiated as soon as possible in order to prevent the spread of multidrug resistance if the percentage of treatment failure is ≥ 10%, as assessed in therapeutic efficacy studies (WHO, 2010).

SURVEILLANCE IN COUNTRIES WHERE TRANSMISSION INTENSITY IS DECREASING

Malaria transmission has decreased in recent years, with the scaling-up of vector control, the introduction of ACTs and other malaria control strategies. In some countries, malaria control programmes cannot find adequate numbers of cases for enrolment in therapeutic efficacy studies at sentinel sites, and some programmes have broadened the eligibility criteria (i.e. age and minimum level of parasitaemia) (WHO, 2009a). Countries that are in pre-elimination or elimination stages must initiate active case detection, with hospitalization of cases until symptoms resolve and follow-up for at least 28 days. If it is still not feasible for a country to conduct therapeutic efficacy studies because of insufficient numbers of patients, surveillance should be based on other methods. For example, molecular markers can be used if they are established and validated (i.e. for chloroquine, mefloquine, and sulfadoxine–pyrimethamine). Similarly, in vitro tests can be used if the threshold for in vitro resistance has been validated. In some circumstances, however, drug efficacy and drug resistance cannot be monitored because of a lack of appropriate methods and the decreasing prevalence of malaria.

2.2 In vitro tests

DEFINITION AND SCOPE

In vitro assays are used to monitor drug resistance by measuring the intrinsic sensitivity of *P. falciparum* parasites to antimalarial drugs. Parasites are exposed to a precise concentration of drug and observed for inhibition of maturation into schizonts. The results of in vitro studies can complement the findings of therapeutic efficacy studies and can provide useful information on the epidemiology of drug-resistant malaria. In view of their technical complexity and cost, it is generally recommended that in vitro tests be conducted in only a small number of reference laboratories in malaria-endemic countries, in order to optimize limited equipment and resources and ensure standardization of methods. For these reasons, national malaria control programmes and research programmes should ideally share access to reference laboratories.

From a research perspective, in vitro tests have several advantages. They offer a more objective approach to determining parasite resistance, as they are based on direct contact between parasites and incremental drug concentrations. Unlike therapeutic efficacy studies, in vitro studies obviate the risk that host factors will confound the results. In addition, several tests can be carried out with the same sample, and several drugs can be studied at the same time, including drugs that are still at the experimental stage (with the exception of prodrugs). Box 4 gives a summary of the applications of in vitro tests for epidemiological monitoring of antimalarial drug resistance (Basco, 2007).

LIMITATIONS AND INTERPRETATION OF IN VITRO STUDIES

The usefulness of in vitro study results for monitoring drug-resistant malaria is limited mainly because of the use of many different tests and methods, which are not always comparable. The tests include the WHO mark III test, the radioisotopic test, enzyme-linked immunosorbent assays with antibodies directed against *Plasmodium* lactate dehydrogenase or histidine-rich protein and the fluorometric assay with DNA-binding fluorescent dyes. As each test has a different end-point (such as the appearance of schizonts with at least three nuclei, a fixed incubation period, an optical density reading in control wells) and different measures of metabolism (incorporation of nucleotide precursor or fluorescent dye, production of parasite-specific enzyme, secretion of soluble antigen), interpretation of the data depends on which test was used (Basco, 2007).

Further, even when the same test is used, the results are often difficult to compare because of differences among laboratories. Results, usually expressed as the 50% inhibitory concentration (IC_{50}), IC_{90} or the minimum inhibitory concentration, are the product of more than 10 different factors (e.g. erythrocyte volume fraction, initial parasitaemia, volume distributed in wells), which are rarely identical across laboratories and over time. Without a standard protocol for conducting in vitro assays of drug sensitivity for field monitoring of drug-resistant malaria, results obtained at different study sites cannot easily be compared. Results should be expressed as a geometric mean (mean IC_{50}, IC_{90} or minimum inhibitory concentration) rather than as percentage in vitro resistance. Geometric means allow more precise comparisons of sites in a country and over time. Furthermore, the in vitro thresholds for several antimalarial medicines, including artemisinins, and several in vitro tests have not been correctly validated (Basco, 2007).

The evidence for a correlation between the results of therapeutic efficacy studies and in vitro tests is inconsistent, due to non-adherence to a standard method or lack of validation of the threshold of resistance in vitro. An infection may be due to two or more parasite populations, as is common in high-transmission

settings, and a predominantly sensitive parasite population might mask the phenotype of a population of resistant parasites present in the isolate. A resistance threshold is validated when an in vitro study and a therapeutic efficacy study are conducted on a population sample of non-immune children or travellers, and the results are compared. The therapeutic efficacy study must have included adequate patient follow-up, and there must be confirmation that treatment failures are due to drug resistance and not to insufficient drug absorption, reinfection or other factors. Once established, a threshold for resistance in vitro applies only to the test used to determine the threshold.

In vitro studies are complex, and current methods have inherent methodological weaknesses. For example, parasites that are sampled from patients recently treated with antimalarial medicines will most likely fail to grow or will grow poorly with a diminished IC_{50}, due to the presence of drugs in the blood (Basco et al., 2002). This is a practical constraint, which disqualifies many samples. Similarly, if the radioisotope method is used, samples with low rates of parasitaemia (< 0.1%) should be excluded. Techniques involving monoclonal antibodies are sensitive enough to detect a parasitaemia of 0.01%. The sensitivity of fluorescence assay such as Sybr green I is being investigated, but high background noise due to the presence of human DNA could limit its usefulness for testing clinical samples (Vossen et al., 2010).

BOX 4. IN VITRO STUDIES FOR MONITORING ANTIMALARIAL DRUG RESISTANCE

Cross-resistance studies

- detect resistance among drugs that belong to the same chemical family or that have similar modes of action;
- detect negative correlations (i.e. one drug is more active when another has less activity in vitro).

Baseline drug sensitivity tests

- establish baseline data on drug sensitivity before new treatments are introduced in national policy;
- when incorporated as a routine component of long-term surveillance, can be used to identify changes over time, especially when the resistance threshold has not yet been determined in vitro;
- can be used for individual sensitivity analysis of each of the medicines in combination therapy.

Temporal and spatial monitoring of parasite drug susceptibility

- provide early warning of impending resistance to first- and second-line treatment before it is clinically apparent;
- reveal changes in sensitivity to drugs that have been withdrawn;
- compare the sensitivity of strains at different sites in the same or different countries (if identical protocols are used).

Note: Despite strict adherence to a standardized protocol, a long follow-up might raise problems of data comparability; investigators may encounter variations in some parameters (human serum batch, pretreated plate batch, drug batch), which are beyond their control.

Validation of molecular markers

- studies with clones and fresh isolates allow detection of a strong correlation between molecular marker and in vitro data.

Note: If there is strong correlation, molecular markers are more convenient, in particular for drugs that require special in vitro test conditions or when the results are difficult to reproduce (pyrimethamine, cycloguanil, sulfadoxine).

In vitro tests have also been used to determine whether two drugs act synergistically, additively or antagonistically against reference clones. Multiple combinations of drugs at different concentrations are required, and the sensitivity of the clone to both drugs must be determined in advance. In vitro tests are not, however, the best means for studying a fixed-dose combination such as sulfadoxine–pyrimethamine or ACTs, as the selected fixed ratio of each component does not allow simulation of drug interactions at varying ratios of concentrations of the two drugs in blood over time.

In vitro tests can provide additional information to confirm the presence of drug-resistant parasites and could be important for monitoring some drugs, especially to detect trends over time. These tests are, however, technically complex, and the significant variation in study procedures and reporting of results prevents comparisons across sites and over time. The immediate priorities in research on in vitro tests for monitoring antimalarial drug resistance are validation of drug resistance thresholds and standardization of procedures and analyses.

Several in vitro drug sensitivity assays for *P. vivax* have been reported; however, researchers have yet to identify the best method of determining the drug response of *P. vivax* isolates. Future research should focus on developing high-throughput assays (Basco, 2007).

2.3 Molecular markers

Characterization of the molecular markers of drug resistance is an important aspect of understanding resistance to antimalarial treatment.[2] Once the genetic changes associated with resistance are identified, drug resistance can be confirmed with molecular techniques. Molecular marker studies also have several practical advantages over in vivo and in vitro tests: many isolates can be studied within a short time, and specimens can be collected, stored and transported far more easily than for in vitro tests. Blood samples can be collected on filter paper strips, which can last several months with proper storage.

GENETIC MARKERS OF RESISTANCE

A limited number of genes involved or potentially involved in *P. falciparum* antimalarial drug resistance have been identified: the genes encoding dihydrofolate reductase (*Pfdhfr*), dihydropteroate synthase (*Pfdhps*), the chloroquine resistance transporter (*Pfcrt*), the multidrug resistance 1 protein (*Pfmdr1*), Na^+/H^+ exchanger (*Pfnhe-1*) and cytochrome *b*. While there has been some success in identifying the molecular markers of resistance for many antimalarial medicines, more research is needed to identify those for other antimalarial drugs, in particular artemisinins. Current knowledge about the molecular markers of resistance to various antimalarial treatments is summarized below.

Pyrimethamine and cycloguanil

Studies of the *Pfdhfr* gene have consistently demonstrated the importance of a point mutation at the Ser108Asn codon in the pyrimethamine-resistant phenotype of *P. falciparum*. Additional point mutations at the Asn51Ile, Cys59Arg and Ile164Leu positions strengthen the resistance of *P. falciparum* to antifolates. The level of resistance increases with the number of mutations (Basco & Ringwald, 2000). Cycloguanil resistance appears to be associated with the double mutations Ser108Thr and Ala16Val (Hyde, 2007).

[2] Molecular markers can also be used to distinguish between reinfection and recrudescence in therapeutic efficacy studies. The techniques for genotyping parasite populations during clinical trials, in order to distinguish between reinfection and recrudescence in therapeutic efficacy studies, have been described (WHO, 2008a). This document covers the six basic aspects of molecular typing: sampling scheme, methods of blood sampling and sample storage, genotyping strategy, analyses and outcome classification, quality control and genotyping of *P. vivax*.

In South America, a mutation at codon 59 is less common and is replaced by the Cys50Arg mutation and repeated insertion of five amino acids between codons 30 and 31 (Bolivia repeat). This repeat insertion is found in association with Ile164Leu and leads to treatment failure (Bacon et al., 2009). Another mutation associated with the Ile164Leu mutation was found in Thailand. Parasites bearing a *Pfdhfr* 164 mutation have significantly higher copy numbers of *gch1* encoding for GTP-cyclohydrolase I, an enzyme that is part of the folate and biopterin biosynthesis pathways. This amplification could represent an adaptation to reduced enzyme activity later in the folate pathway (Nair et al., 2008).

Sulfadoxine

The mechanism of resistance to sulfadoxine has been associated with five point mutations, at the Ser436Ala/Phe, Ala437Gly, Lys540Glu, Ala581Gly and Ala613Thr/Ser codons of the *Pfdhps* gene. The mutations at 437 and 540 confer some degree of resistance; the 436, 581 and 613 mutations all contribute to a higher degree of resistance (Hyde, 2007).

Sulfadoxine–pyrimethamine

In antimalarial chemotherapy, sulfadoxine is always combined with pyrimethamine. The antimalarial activity of this combination is based on the specific inhibition of two successive enzymes in the biosynthesis of folic acid, with subsequent synergistic action. Several mutations in both the *Pfdhfr* and *Pfdhps* genes are necessary to induce treatment failure with the sulfadoxine–pyrimethamine combination, such as triple mutations at codons 108, 51 and 59 of the *Pfdhfr* gene and double mutations at codons 437 and 540 of the *Pfdhps* gene (Kublin et al., 2002). In population studies, mutations at codon 59 of the *Pfdhfr* gene and codon 540 of the *Pfdhps* gene are strongly predictive of treatment failure. A quintuple genetic mutation may create the conditions needed for the emergence of the *Pfdhfr* Ile164Leu mutation and the *Pfdhps* Ala581Gly mutation (Lynch et al., 2008).

The relation between parasite genotype and therapeutic response to sulfadoxine–pyrimethamine is influenced by parasite, pharmacokinetics and human factors. When a parasite has wild-type *Pfdhfr* without a mutation, the risk for failure is trivial, regardless of the *Pfdhps* alleles. In contrast, the risk increases with the number of mutations in the *Pfdhfr* gene, particularly when there is an additional mutation in the *Pfdhps* gene or when immunity is lacking (Sibley et al., 2001; Bacon et al., 2009). Cumulative mutations in the *Pfdhfr* gene increase parasite clearance time and the risk for gametocyte carriage. As a result, even though sulfadoxine–pyrimethamine remains effective, the emergence of one or two mutations can increase the transmission of malaria and the spread of resistance (Mendez et al., 2002).

4-Aminoquinolines

The *Pfcrt* gene is situated on chromosome 7 and encodes a transport protein in the vacuolar membrane. This gene plays a major role in determining the phenotype of chloroquine resistance, when lysine is replaced at codon 76 by threonine. This mutation is associated with different sets of mutations at other codons, most commonly Cys72Ser, Met74Ile, Asn75Glu, Ala220Ser, Gln271Glu, Asn326Ser, Ile356Thr and Arg371Ile, although the specific set of accompanying mutations depends on the geographical setting.

The *Pfmdr1* gene, which is situated on chromosome 5 and codes for the P-glycoprotein homologue 1, has also generated interest in the context of chloroquine resistance. The mutations to *Pfmdr1* that have been associated with chloroquine resistance include Asn86Tyr, Tyr184Phe, Ser1034Cys, Asn1042Asp and Asp1246Tyr. Linkage disequilibrium between the Lys76Thr mutation on the *Pfcrt* gene and the Asn86Tyr mutation on the *Pfmdr1* gene has been observed in field studies. Highly chloroquine-resistant isolates

appear to have at least the Lys76Thr and Ala220Ser mutations in the *Pfcrt* gene and are generally associated with the Asn86Tyr mutation in the *Pfmdr1* gene. *Pfmdr1* mutations probably do not confer resistance to chloroquine but have an important modulatory effect (Roepe, 2009).

Some chloroquine-resistant isolates have shown cross-resistance with amodiaquine both in vivo and in vitro. *Pfcrt* and *Pfmdr1* alleles interact to yield different levels of resistance to chloroquine and amodiaquine. The *Pfcrt* mutations at codons 72–76 observed in South America are associated with high levels of amodiaquine resistance, whereas *Pfcrt* mutations in South-East Asia and Africa are linked to greater resistance to chloroquine and moderate resistance to amodiaquine. This difference may be due to the extent of previous use of amodiaquine in different regions. Amodiaquine resistance may also be modulated by the *Pfmdr1* mutations Asn86Tyr and Asn1042Asp (Sá et al., 2009).

Amino-alcohols

The *Pfmdr1* gene has also been implicated in resistance to amino-alcohols and to artemisinins. Studies conducted in the Greater Mekong subregion (Cambodia and Thailand) showed that increases in copy numbers of this gene are responsible for resistance to mefloquine and to increased risks for treatment failure with artesunate–mefloquine and artemether–lumefantrine (four-dose regimen only) (Price et al., 2004; Price et al., 2006). In vitro susceptibility to mefloquine, quinine, halofantrine and artemisinin increased when the *Pfmdr1* copy numbers were reduced or when the parasites carried *Pfmdr1* mutations (Sidhu et al., 2006; Nkhoma et al., 2009). In South-East Asia, the presence of the Asn86Tyr mutation is a negative marker for gene amplification. *Pfmdr1* amplication and deamplification are relatively frequent events related to the rapid evolution of mefloquine resistance when the drug is used as monotherapy.

In several field studies, artemether–lumefantrine appeared to select for the wild-type *Pfmdr1* Asn86 allele in recurrent infection, which could be a marker for reduced susceptibility to lumefantrine (Sisowath et al., 2005; Dokomajilar et al., 2006; Happi et al., 2009).

Quinine

It is difficult to demonstrate resistance to quinine. *P. falciparum* chromosome 13 contains a candidate gene (*Pfnhe-1*), which encodes a putative Na^+/H^+ exchanger (Ferdig et al., 2004). Repeat polymorphism in *Pfnhe-1* microsatellite ms4760 was significantly associated with a low quinine response, but additional field studies are needed to validate this marker (Henry et al., 2009; Andriantsoanirina et al., 2010; Okombo et al., 2010). Studies with clones and field isolates indicate that the Asn86Tyr, Ser1034Cys, Asn1042Asp and Asp1246Tyr mutations may be associated with decreased susceptibility to quinine (Reed et al., 2000; Sidhu, Valderramos & Fidock, 2005). Like the response to chloroquine, that to quinine is influenced by mutations in several transporter genes (*Pfcrt*, *Pfmdr1* and *Pfnhe-1*) (Ekland & Fidock, 2007).

Artemisinins

Artemisinin resistance has been induced in human and murine models and observed in clinical trials at the Cambodia–Thailand border (Noedl et al., 2008; Dondorp et al., 2009). In falciparum malaria, artemisinins are thought to inhibit the sarco-endoplasmic reticulum calcium-ATPase (SERCA)-type, PfATPase 6 protein (Woodrow & Krishna, 2006); however, this is unlikely to be the sole target (Valderramos et al., 2010b). One molecular marker for artemether resistance has been proposed, *PfATPase6* Ser769Asn, but this suggestion is based exclusively on findings from in vitro tests (Jambou et al., 2005), and field studies have not confirmed this hypothesis (Zhang et al., 2008; Tahar, Ringwald & Basco, 2009). Amplification of the *Pfmdr1* gene is associated with relatively small but significant reductions in susceptibility to artemisinins in vitro, which

could explain the cross-resistance observed between amino-alcohols and artemisinins in vitro (Price et al., 2004; Chavchich et al., 2010). So far, none of the known markers, in particular *Pfmdr1* copy numbers or mutations, *PfATPase6*, the 6-kb mitochondrial genome (including cytochrome *b*, *COXI* and *COXIII*) or *Pfubp-1* encoding a deubiquitinating enzyme, correlate with the artemisinin resistance phenotype observed at the Cambodia–Thailand border (Imwong et al., 2010).

Atovaquone

Molecular analysis of recrudescent isolates has demonstrated that atovaquone resistance is linked to a single mutation at the cytochrome *b* gene codon (Tyr268Asn, Tyr268Ser or Tyr268Cys), inducing an approximately 1000-fold increase in the IC_{50} for atovaquone. In previous studies, a single mutation at cytochrome *b* was observed to compromise the efficacy of this medicine (Musset et al., 2006a).

INDICATIONS AND LIMITS

Like in vitro tests, molecular markers of resistance can be used to provide early warning in geographical or temporal monitoring. Molecular markers are useful for monitoring the prevalence of mutations after a drug has been withdrawn or when a drug combination is used (Kublin et al., 2003). They can replace in vitro tests for antifolates, which have several technical difficulties (requirement for special medium, poor solubility in water or usual solvents). They are particularly useful for providing direct evidence that a treatment or prophylaxis failure is a result of selection of resistant parasite populations.

Although the identification of molecular markers raised hopes of a predictive test of treatment efficacy, several challenges remain (Mbacham & Njikam, 2007). Different methods of varying sensitivity are used in different laboratories (Färnert et al., 2001; Ranford-Cartwright et al., 2002). Mixed infections are common in many endemic areas, and resistant parasite populations might be masked by sensitive populations, especially if the method of detection is not sensitive. This may lead to discordant results between therapeutic efficacy and molecular marker studies. In order to be useful for public health, molecular markers must consistently and reliably predict patients' clinical and parasitological outcomes (Hastings, 2007). Finally, successful use of molecular markers for monitoring antimalarial drug resistance requires close collaboration between national malaria control programmes and research institutions.

2.4 Measurement of drug concentrations

Pharmacokinetic studies are used to characterize drug absorption, distribution, metabolism and elimination in the body. They also demonstrate the factors that influence blood concentrations, and their results indicate the appropriate doses of a medicine. Once the pharmacokinetics of a drug is understood, the dose can be adapted to different populations. As the pharmacokinetics of antimalarial drugs varies widely, there are wide variations in blood concentration profiles, which increase in patients who do not adhere to a full treatment course or who absorb the drug poorly.

One of the main aims of drug efficacy monitoring is to distinguish between treatment failure due to resistance and that due to other causes, such as inadequate concentration of a drug in the blood. A correct interpretation of treatment failure is particularly important when treatments are expected to yield 95% cure rates and when failures are rare in clinical trials. If treatment fails, the drug concentration in the patient is likely to be less than the minimum inhibitory concentration for proliferating parasites. A very low drug concentration at the time of failure does not, however, necessarily mean that the strain is sensitive, as the blood sample might have been taken some days after reappearance of clinical signs, and the malaria itself

might modify the volume of distribution of the drug, with a resulting reduction in its blood concentration. The finding that the blood concentration is higher than the minimum inhibitory concentration generally sufficient to eliminate sensitive parasites is a strong argument for resistance.

Population pharmacokinetic studies address the sources and correlates of variability in drug concentrations in the target population receiving clinically relevant doses of a drug. Models of population pharmacokinetics can be used to characterize pharmacokinetics from limited data. If such studies have been conducted, a limited number of blood samples is sufficient to determine, with the appropriate software programs and models, the pharmacokinetics of the drug and the characteristics in individuals that give rise to interindividual variation (population-based method of analysis) (Simpson, Aarons & White, 2001). Further research on the pharmacokinetics of antimalarial medicines is needed, as the minimum inhibitory concentrations have not yet been determined for all treatments.

Blood concentration studies provide key evidence for determining whether a malaria-resistant strain is present and should therefore be part of in vivo evaluations, provided that there is sufficient laboratory capacity. No special storage conditions are required, except for artemisinins. A single drug measurement, typically on day 7, can provide a reasonable estimate of exposure (White et al., 2008). Simple field-adapted assay methods are being developed, which will require only a small volume of capillary blood dried on filter paper. As new methods become available, national malaria control programmes and other organizations conducting therapeutic efficacy studies should incorporate drug concentration studies into routine monitoring of therapeutic efficacy, if funding is available.

The interpretation of the results of blood concentration studies for determining drug resistance is not, however, always straightforward. For example, in therapeutic efficacy studies that included sampling of plasma or whole blood at various times during follow-up, it was found that children had lower drug concentrations than adults, and cured persons had higher drug concentrations than those who failed treatment (Aubouy et al., 2003; Barnes et al., 2006; Price et al., 2007). One explanation for this finding might be that the failures were due to inadequate drug concentrations, rather than resistance; however, they may also indicate partial resistance and survival of strains in patients with reduced plasma drug concentrations.

3. Global review of antimalarial drug efficacy and drug resistance

3.1 *Plasmodium falciparum*

The global review of the efficacy of antimalarial drugs against *P. falciparum* presented in this section is based on the studies in the WHO global database on antimalarial drug efficacy. For each drug, the results of the efficacy studies are described first, complemented by data from in vitro and molecular marker studies and assessments of pharmacokinetics. The WHO database is described in detail in Annex 1.

The database contains 3932 studies representing 267 841 patients who participated in studies between 1996 and June 2010. Studies that met the following criteria were included in the analysis:

- conducted between 2000 and June 2010 with a follow-up of ≥ 28 days,
- a minimum sample size,
- PCR correction in studies conducted in high-transmission areas and
- outcomes classified as either adequate clinical and parasitological response or treatment failure.

In addition, only studies of the following treatment regimens were included:

- an ACT with a regimen that included 3 days of artemisinin[3] or an artemisinin derivative;
- a combination such as chloroquine–sulfadoxine–pyrimethamine, amodiaquine–sulfadoxine–pyrimethamine, quinine–antibiotic (over 7 days) or atovaquone–proguanil; or
- an antimalarial medicine that is part of an ACT or one of the other combinations listed above, used as monotherapy.

When studies conducted in high-transmission areas gave results by age group, the results for children under 5 years of age were used in the analysis. All studies with an inclusion criterion of either 'fever' or 'history of fever' were included. If the methods used differed significantly from the standard protocol (e.g. in patient inclusion criteria, classification of patient outcome), they were not included in the final analysis. Studies which included patients who were known to be infected with HIV, pregnant or had severe malaria were also excluded.

The analysis is based on data from 1120 studies representing 81 848 patients. Of the studies that were included, 94.4% were conducted with the 2001 (n = 1006) or 2009 WHO protocol (n = 51). The others either followed the 1973 WHO protocol (n = 34) or did not follow a standard protocol but had methods that made them appropriate for inclusion (n = 29).

The treatment failure rates observed during the therapeutic efficacy studies that were eligible for inclusion in the analysis are presented in Table A1.2 by WHO region and country and in Table A1.3 by antimalarial medicine. The percentage of treatment failures on day 28 (calculated by the per-protocol method) is equal to the total number of early treatment failures, late clinical failures and late parasitological failures, divided by the total number of patients who completed the study follow-up, multiplied by 100. The median, minimum and maximum describe the range of treatment failures observed in the studies. If the treatment failure on day 28 was not available, the treatment failure on another day (e.g. day 35, 42 or 63) was used, and the alternative day is given between brackets. The number of studies and the years during which the studies were conducted are also shown.

[3] In this section, 'artemisinin' refers to the final pharmaceutical product.

MONOTHERAPIES

In view of the risks for treatment failure due to the emergence of drug resistance, monotherapies for the treatment of uncomplicated malaria are no longer recommended. Over the past decade, most malaria-endemic countries have shifted their national treatment policies to ACTs, and fewer data are available on the efficacy of monotherapies. Nonetheless, studies of the efficacy of a single drug that is now used as part of a combination therapy provide potential information about the overall efficacy of ACTs. Studies of oral artesunate monotherapy being conducted in research settings can help to improve our understanding of resistance to artemisinin and its derivatives.

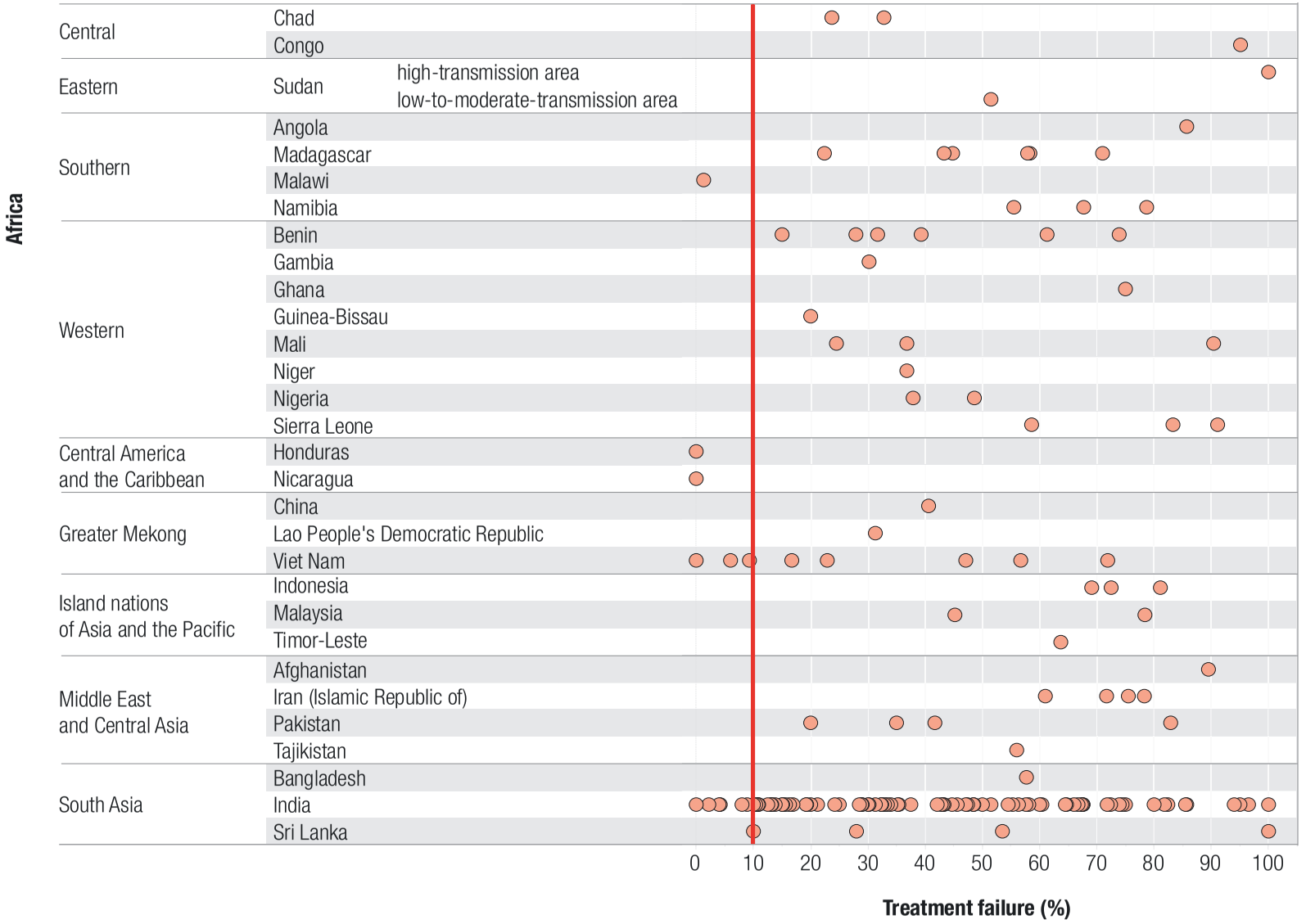
Chloroquine

The studies of chloroquine efficacy included in the analysis (n = 153) were conducted in 30 countries between 2000 and 2009 (Tables A1.2 and A1.3). The median treatment failure rates were high to extremely high (19.8–100%) in all the countries except Honduras, Malawi and Nicaragua (0–1.3%) (Figure 1). Chloroquine remains effective only in Central America, where clinical studies in Honduras and Nicaragua have confirmed its 100% efficacy. Molecular studies are currently under way to confirm the absence of parasites carrying *Pfcrt* and *Pfmdr1* mutations in these two countries. *Pfcrt* mutant parasites have recently been detected in Haiti; however, therapeutic efficacy studies are still needed to determine the prevalence of chloroquine treatment failure in the population (Londono et al., 2009). In India, monitoring of chloroquine continued until 2008 despite consistently high failure rates (Valecha et al., 2009).

Since the withdrawal of chloroquine, there have been signs of regression of chloroquine resistance in some areas. In China and Viet Nam, a significant regression of chloroquine resistance has been documented in in vitro and molecular marker studies, while treatment failure rates remain high (Chen et al., 2008; Yang et al., 2008; Isozumi et al., 2010). In Kenya and Malawi, where there is a high level of transmission and almost exclusively infection with *P. falciparum*, there have been signs of a reduction in the prevalence of chloroquine-resistant parasites. Chloroquine was withdrawn from the market in Malawi in 1993 and in Kenya in 1999, when the treatment policy in both countries changed to ACTs. In Malawi, fewer than 10 years after its withdrawal, a dramatic re-emergence of chloroquine-sensitive parasites was observed on molecular analysis (Kublin et al., 2003; Mita et al., 2003). Chloroquine was subsequently shown to have 99% curative efficacy in children with uncomplicated malaria (Laufer et al., 2006). The prevalence of a mutant *Pfcrt* gene at codon 76 fell considerably, as did evidence of resistance in vitro (Wilson et al., 2005; Nkhoma, Molyneux & Ward, 2007). In Kenya, a reduction in resistance to chloroquine was also observed in vitro and with molecular markers, although at a slower rate (Mwai et al., 2009). It is noteworthy that the prevalence of the Asn86Tyr mutation in the *Pfmdr1* gene has not regressed at the same rate as mutation in the *Pfcrt* gene.

While these results are interesting, caution is still required. The disappearance of parasites carrying the mutant *Pfcrt* gene may be linked to expansion of wild-type parasites still present in the subpopulation replacing the mutant parasites, rather than a reversal of the Lys76Thr mutation (Mita et al., 2004; Ariey et al., 2006; Laufer et al., 2010). Widespread reintroduction of chloroquine is not recommended, as it is still too early to predict how long it might take for chloroquine resistance to reappear or to be reintroduced from neighbouring regions.

FIGURE 1. Treatment failure rates with chloroquine by subregion (2000–2009)



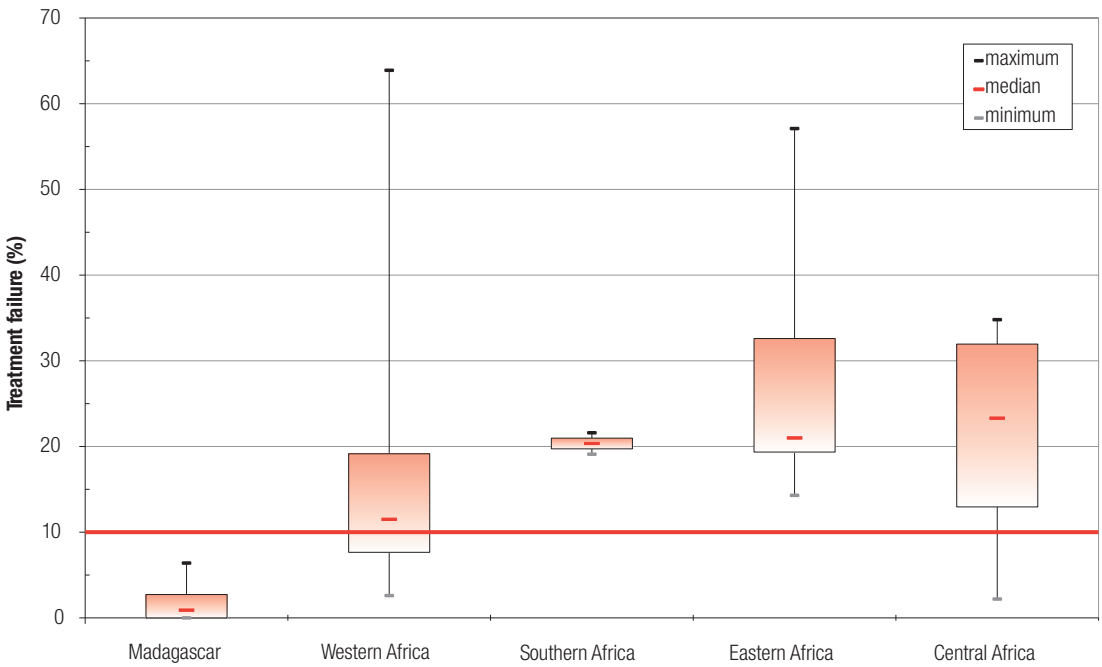
The definition of each subregion is given in Annex 2. Each circle represents a study with a 28-day follow-up. The figure does not distinguish between variations in sample size, protocol year or study location. The red line indicates the threshold of 10% of treatment failure at which countries should initiate a change of the antimalarial medicine recommended in their malaria treatment policy.

Amodiaquine

Despite cross-resistance between chloroquine and amodiaquine, amodiaquine is more effective than chloroquine in areas with identified chloroquine-resistance. Amodiaquine was therefore chosen by several countries as the first-line drug in combination with artesunate. The rate at which amodiaquine loses its efficacy appears to depend on the type of mutations linked to chloroquine resistance and on previous intensive use of amodiaquine in the population (Sá et al., 2009).

The 59 studies of amodiaquine efficacy included in the analysis were conducted in 23 countries between 2000 and 2007 (Tables A1.2 and A1.3). The efficacy of amodiaquine is heterogeneous over the African continent, but the median treatment failure rate was lower in western Africa than in the other subregions (Figure 2). In South America and the Middle East, the median treatment failure rate was very high, ranging from 28.8% to 53.1%.

FIGURE 2. Treatment failure rates with amodiaquine in African subregions and Madagascar (2000–2007)

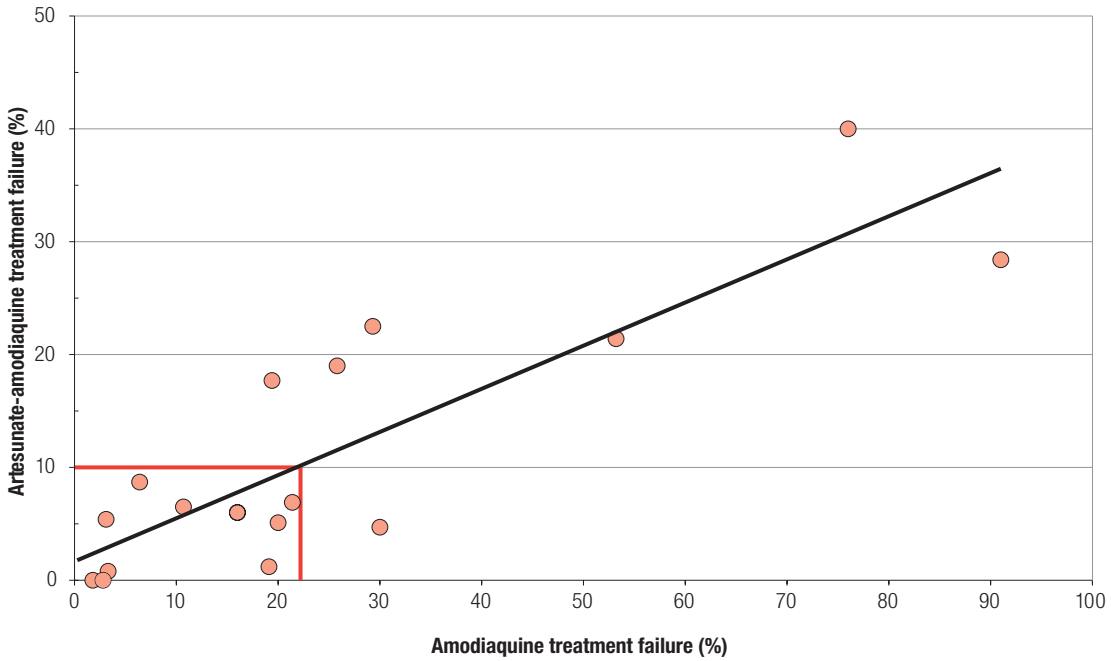


The box plots depict the following summary statistics for the efficacy studies conducted for each subregion or country and drug with a follow-up of 28 days: the minimum value (bottom whisker), the 25th percentile, the median value, the 75th percentile and the maximum value (upper whisker). Subregions or countries are sorted by median values, in ascending order. The box plots do not illustrate variations in sample size, protocol year or study location. The red line indicates the threshold of 10% of treatment failure at which countries should initiate a change of the antimalarial medicine recommended in their national malaria treatment policy.

In areas where artesunate resistance is low or absent, the therapeutic efficacy of artesunate–amodiaquine is highly correlated with the efficacy of amodiaquine alone. This is illustrated in Figure 3, which is based on an analysis of 19 studies in which amodiaquine was compared with artesunate–amodiaquine (r^2 = 0.73). As the treatment failure rate of amodiaquine reaches 22%, the failure rate of the

artesunate–amodiaquine combination is likely to exceed 10%, the threshold above which WHO recommends to initiate a change in treatment policy (see red lines in Figure 3). An amodiaquine failure rate > 22% has been observed in 10 African countries, some of which are currently using artesunate–amodiaquine as the first-line drug (Burkina Faso, Cameroon, the Congo, the Democratic Republic of the Congo, Gabon, Liberia, Sierra Leone, the Sudan – high-transmission area). Parasite strains that are highly resistant to amodiaquine have been reported in the United Republic of Tanzania, which could further compromise the use of artesunate–amodiaquine in Africa (Sá et al., 2009).

FIGURE 3. Correlation between the total failure rates of amodiaquine and artesunate–amodiaquine

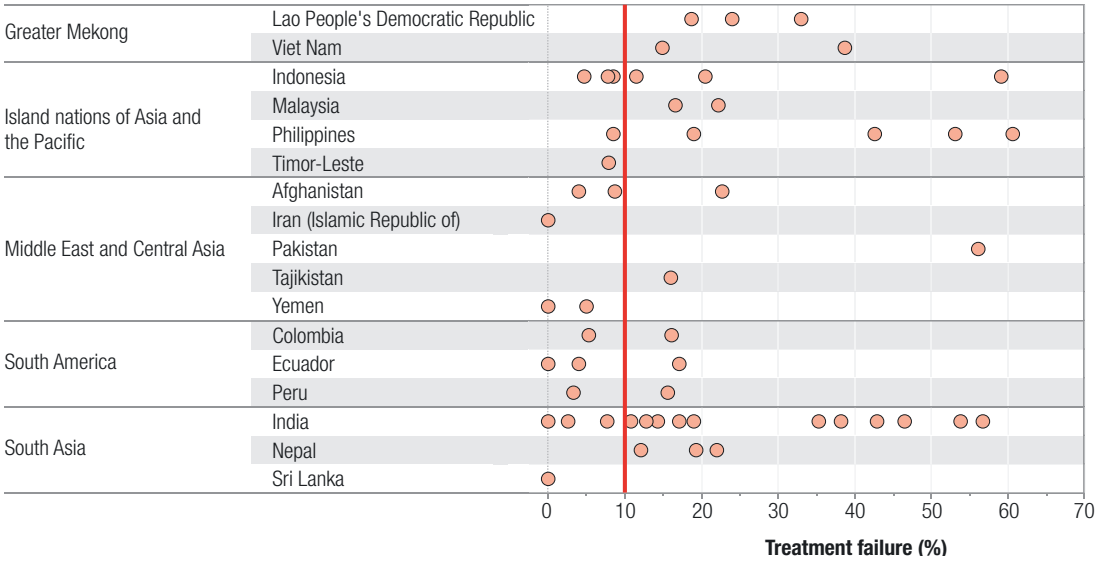


Sulfadoxine–pyrimethamine

Although sulfadoxine-pyrimethamine is actually a co-formulation of two different medicines, it is considered as a monotherapy because the two components act on the same biosynthesis pathway of the parasite. Sulfadoxine–pyrimethamine has been widely used to treat chloroquine-resistant malaria. The 162 studies of its efficacy included in the analysis were conducted in 45 countries between 2000 and 2007 (Tables A1.2 and A1.3). The treatment failure rate of this combination remains low in several countries of South America and the Middle East and Central Asia, with median values of 4.7% and 5%, respectively (Figure 4).

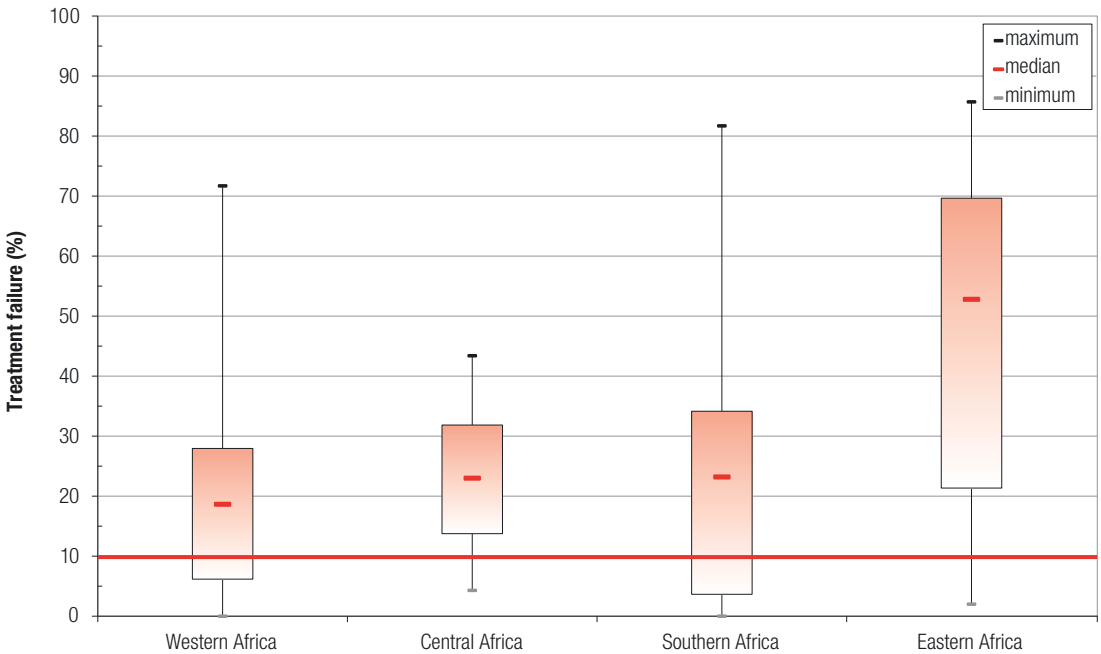
The median failure rate in eastern Africa (52.8%) is higher than in the western (18.7%), central (23.0%) and southern (23.2%) subregions (Figure 5). Studies with molecular markers have confirmed rapid development of resistance after the drug was used at national level in a number of settings (Malisa et al., 2010; Sridaran et al., 2010).

FIGURE 4. Treatment failure rates with sulfadoxine–pyrimethamine by subregion (2000–2007)



The definition of each subregion is given in Annex 2. Each circle represents a study with a 28-day follow-up. The figure does not distinguish between variations in sample size, protocol year or study location. The red line indicates the threshold of 10% of treatment failure at which countries should initiate a change of the antimalarial medicine recommended in their malaria treatment policy.

FIGURE 5. Treatment failure rates with sulfadoxine–pyrimethamine in African subregions (2000–2007)



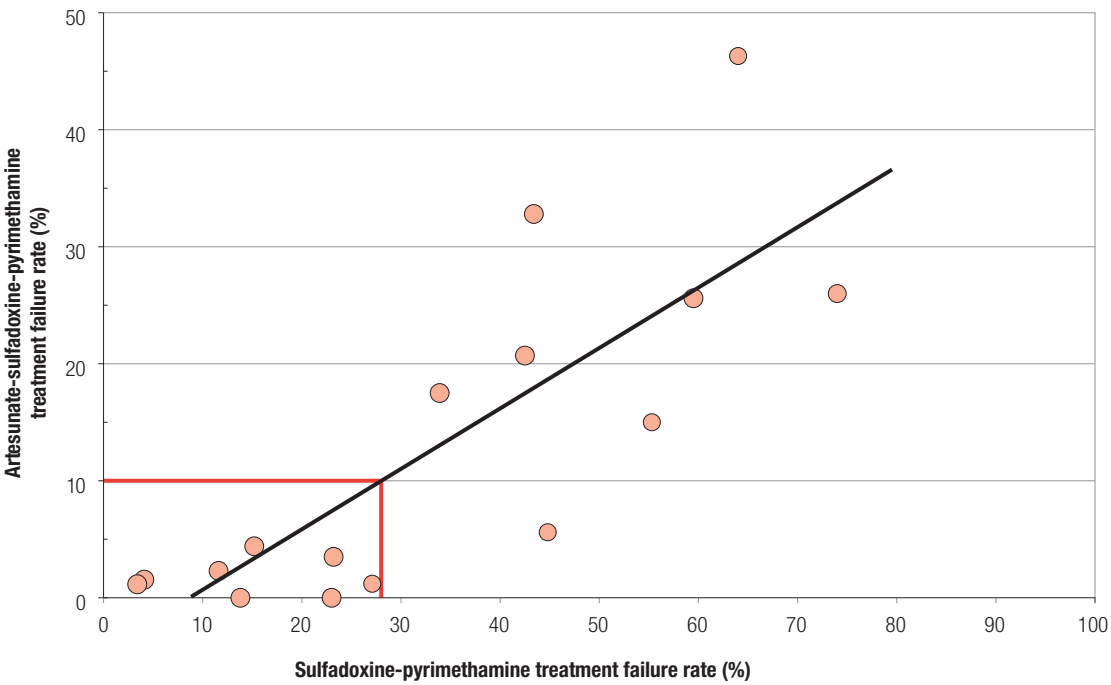
The box plots depict the following summary statistics for the efficacy studies conducted for each subregion and drug with a follow-up of 28 days: the minimum value (bottom whisker), the 25th percentile, the median value, the 75th percentile and the maximum value (upper whisker). Subregions are sorted by median values, in ascending order. The box plots do not illustrate variations in sample size, protocol year or study location. The red line indicates the threshold of 10% of treatment failure at which countries should initiate a change of the antimalarial medicine recommended in their national malaria treatment policy.

As observed with amodiaquine, the efficacy of artesunate–sulfadoxine–pyrimethamine is correlated with the efficacy of sulfadoxine–pyrimethamine alone (r^2 = 0.65, n = 16). As shown in Figure 6, as the treatment failure rate of sulfadoxine–pyrimethamine reaches 28%, the failure rate of the artesunate–sulfadoxine–pyrimethamine combination is likely to exceed 10%. In six studies in India and one in Pakistan, this threshold was largely exceeded (Figure 4).

In contrast to the situation with chloroquine, resistance to antifolates emerged rapidly, after only 1–2 years of intensive use. Moreover, reductions in resistance have been reported, although they are rare and poorly documented. The absence of reduction may be a result of cross-resistance between sulfadoxine–pyrimethamine and antibiotics such as co-trimoxazole or the existence of compensatory mutations in resistant parasites (Laufer & Plowe, 2004). Furthermore, sulfadoxine–pyrimethamine is still circulating in large quantities in the informal sector, which keeps up drug pressure on the regional parasite populations.

After 2 years of use of insecticide-treated nets in a village in the United Republic of Tanzania, the prevalence of wild-type strains was higher than in a nearby control village (Alifrangis et al., 2003). Confounding and other factors, such as migration of sensitive parasites into study sites, weaken the conclusions of such studies (Hastings, Nsanzabana & Smith, 2010). In Peru, the frequency of mutations conferring sulfadoxine–pyrimethamine resistance appeared to decline between 1997 and 2006; however, the studies were not conducted at exactly the same sites and in the same epidemiological setting; e.g. a study in 1997 was done during an epidemic outbreak (Kublin et al., 1998; Zhou et al., 2008). Additional field studies are needed to confirm the regression of antifolate resistance (Talisuna et al., 2003).

FIGURE 6. Correlation between total failure rates of sulfadoxine–pyrimethamine and artesunate–sulfadoxine–pyrimethamine



Because of its long half-life, low cost and safety in pregnant women and children, sulfadoxine–pyrimethamine has been used for intermittent preventive treatment of malaria in infants and pregnant women living in areas of high transmission. This treatment targets asymptomatic persons, usually with low parasite densities. Sulfadoxine–pyrimethamine remains effective for intermittent preventive treatment, even in areas with moderate resistance (ter Kuile, van Eijk & Filler, 2007; Aponte et al., 2009), but use of this combination is ineffective when highly resistant strains emerge (Gosling et al., 2009). As monitoring the therapeutic efficacy of sulfadoxine–pyrimethamine is unethical in many settings because of its high failure rate, molecular marker studies are needed to monitor the trend in the prevalence of parasites resistant to sulfadoxine–pyrimethamine, in particular in Africa, where several countries have implemented intermittent preventive treatment (WHO, 2009b). The location and prevalence of mutations of *Pfdhfr* and *Pfdhps* in parasites sampled in Africa are available at http://www.drugresistancemaps.org/ipti/.

Mefloquine

Mefloquine resistance appeared at the Cambodia–Thailand border only a few years after its introduction (Boudreau et al., 1982). There are several probable reasons for this rapid onset. Pre-existing strains in the region had markedly reduced sensitivity to quinine. Further, the long half-life of mefloquine may have allowed exposure to subtherapeutic concentrations. Common use of the low-dose, single-dose regimen (15 mg/kg body weight) in this region might also have contributed to the rise in resistance. The *Pfmdr1* gene amplication that confers mefloquine resistance is acquired relatively rapidly. The higher dose of 25 mg/kg body weight that is usually recommended is known to have several adverse effects, in particular vomiting, which may lead to lower blood concentrations and subsequent treatment failure (Simpson et al., 2000).

Between 2000 and 2008, only five studies of mefloquine efficacy were conducted in which a total dose of 25 mg/kg body weight was used and which were eligible for inclusion in the analysis (Tables A1.2 and A1.3). The studies conducted in Benin, Brazil, Guyana, Nigeria and Suriname, showed low treatment failure rates (2.4–8.8%).

Mefloquine resistance continues to be a concern in the Greater Mekong subregion, in particular in Thailand and Cambodia, where artesunate–mefloquine is still used as a first-line treatment. The national malaria control programme in Thailand detected a gradual decline in the efficacy of mefloquine at its sentinel sites, although monitoring of mefloquine monotherapy was last conducted in 2004. Even when the dose was increased from 15 to 25 mg/kg body weight, efficacy increased only temporarily (Rojanawatsirivet et al., 2004). In Cambodia, after implementation of rapid diagnostic tests and replacement of artesunate–mefloquine by dihydroartemisinin–piperaquine in Pailin Province, a reduction in mefloquine resistance was detected using molecular markers. The high 'fitness cost' linked to mefloquine resistance and removal of mefloquine pressure led to deamplification of *Pfmdr1* copy numbers between 2005 and 2007, resulting in a decrease in the treatment failure rate of artesunate–mefloquine (≤5%) in 2007–2008 (Preechapornkul et al., 2009; Dondorp et al., 2009; Imwong et al., 2010).

In Myanmar and Viet Nam, the treatment failure rate was as high as 40% in the late 1990s and early 2000s; however, a low dose of 15 mg/kg body weight was used (Huong et al., 2001; Trung et al., 2001; Smithuis et al., 2004). No recent studies have been reported in which a dose of 25 mg/kg body weight was used.

In Africa, in vitro studies conducted prior to the introduction of mefloquine showed the presence of parasites with reduced sensitivity to mefloquine that were still sensitive to chloroquine (Oduola et al., 1987). Validation of molecular markers for chloroquine and mefloquine resistance now allows better understanding of these results. Recently, isolates with increased *Pfmdr1* copy numbers have been detected in West Africa and have been associated with mefloquine treatment failure in travellers (Witkowski, 2010a; Witkowski 2010b). Efficacy studies conducted in Benin and Nigeria showed low treatment failure rates.

In South America, mefloquine resistance has remained low, although few therapeutic efficacy studies have been performed. The prevalence of an increased *Pfmdr1* copy number was 12% among 93 samples in the Bolivarian Republic of Venezuela in 2003 and 2004 (Griffing et al., 2010).

Quinine

Since the adoption of ACTs as first-line treatment for uncomplicated cases of malaria, quinine has been more commonly used as second-line treatment and remains the drug of choice for pregnant women. Quinine is still used in many countries for the treatment of severe malaria, although artesunate is now recommended for adults. According to the 2010 *Guidelines for the treatment of malaria,* for the treatment of uncomplicated malaria, oral treatment with quinine should be combined with an antibiotic; for severe malaria, injectable quinine should be followed by either oral quinine with antibiotic or artesunate with clindamycin or doxycycline, or a full course of an ACT, once the patient can tolerate oral therapy (WHO, 2010).

Between 2002 and 2007, nine studies were conducted in six countries with quinine 25 mg/kg body weight of quinine base per day alone or in combination with an antibiotic. High failure rates were reported in two studies in the Bolivarian Republic of Venezuela (≥ 20%), but the data were not PCR-corrected (Tables A1.2 and A1.3). Quinine–tetracycline and quinine–doxycycline were 100% effective in Pakistan and Cambodia, respectively. Many studies with significant methodological differences were reported in the literature. The variations in study methods included duration of treatment (3–7 days), quinine dose (15–25 mg/kg body weight of quinine base per day), monotherapy versus combination (with antibiotics or sulfadoxine–pyrimethamine) and patient inclusion criteria (pregnant women, severe malaria). Therefore it is difficult to estimate the efficacy of quinine globally.

In the treatment and follow-up of patients, it is important to bear in mind individual differences in the clinical response to quinine. For example, a temporary rise in parasitaemia may occur shortly after the first dose, suggesting early treatment failure, although this does not tend to affect the treatment outcome (Gachot et al., 1996). In view of its relatively slow action, as seen by the 48-h parasite reduction rate, the length of treatment should be adjusted to the parasite load (White, 1997). In the event of hyperparasitaemia, it may be necessary to extend treatment beyond 7 days or to combine quinine with another antimalarial agent (Edwards & Krishna, 2004).

The methods available for measuring resistance to quinine in vitro are inadequate. The in vitro resistance threshold of 500 nmol/l has not yet been adequately validated (Basco, 2007). Cure requires a continuous concentration of ≥ 6 µg/ml over at least 7 days (Pukrittayakamee et al., 2003). As quinine has a short half-life, a single drug level measurement on day 7 is insufficient, and more frequent sampling during therapy is required. Validation of a molecular marker would be invaluable for the detection of quinine resistance.

Many countries have expressed an interest in studying the efficacy of quinine. Pilot studies conducted by WHO have confirmed that the standard protocol can be used to monitor quinine efficacy, either alone or in combination with antibiotics such as doxycycline or tetracycline, and more studies are encouraged (WHO, 2009a). Most patients experience adverse effects after the third day of treatment, in particular tinnitus, temporary deafness or dizziness. Ideally, evaluation of the efficacy of quinine would require patient hospitalization in order to ensure compliance.

Artemisinin and artemisinin derivatives

In contrast to most previous antimalarial treatments, such as chloroquine and sulfadoxine–pyrimethamine, which are eliminated slowly, artemisinin and its derivatives are eliminated quickly and target all the blood

stages of the malaria life cycle, including early ring forms. This is particularly beneficial for the treatment of severe malaria, when rapid elimination of parasites is critical for patient recovery. Most scientists were confident that artemisinin and its derivatives would become some of the most significant medicines available for the treatment and control of malaria (Bloland, Ettling & Meek, 2000; White, 2008).

Ten studies conducted with oral artemether, artesunate or dihydroartemisinin monotherapy over 5 days in five countries and 18 studies of 7-day oral monotherapy in five countries were included in the analysis (Tables A1.2 and A1.3). More than half (n = 19) the studies were conducted in China and Viet Nam. The median treatment failure rate with artesunate over 5 days in these two countries was 15%, whereas the rate after 7-day treatment was 3%. These findings are consistent with those of clinical trials in the 1970s, when a recrudescence rate of 48% was reported after a 3-day treatment; when treatment was extended to 5 and 7 days, the recrudescence rates decreased to 10% and 2%, respectively (Li et al., 1994). They can be explained by the short half-life of artemisinin and its derivatives, as not all parasites would necessarily be eliminated after the initial rapid effect of a short treatment with oral artemisinin–based monotherapy. Therefore, monotherapy was not effective unless it was administered over an extended time. In the same way that parasites that are consistently exposed to a suboptimal dose of treatment develop resistance, an incomplete or short treatment with oral artemisinin-based monotherapy could also facilitate the development of resistance, although the short half-life of these drugs reduces the time window in which resistant parasites can be selected.

Failures with oral artesunate monotherapy may be due not only to decreased sensitivity but also to high pretreatment parasitaemia (Ittarat et al., 2003). As with quinine, it is not unusual to witness a rise in parasitaemia after the start of treatment with artemisinin-based monotherapies (Silachamroon et al., 2001). Reduced immunity (resulting from HIV infection or splenectomy) and haemoglobin abnormalities can also lead to excessively long delays in treatment response (Yuthavong et al., 1989; Thu et al., 1997; Treeprasertsuk et al., 2000). Resistance to artemisinin and its derivatives is discussed in more detail in section 4.5.

Atovaquone–proguanil

Strictly speaking atovaquone-proguanil is not a monotherapy but classified as such because its efficacy relies on the synergistic action of the two components (WHO, 2001). Early studies of atovaquone administered as a monotherapy showed that resistant parasites were selected rapidly, and the synergistic combination atovaquone–proguanil was developed in order to delay the emergence and spread of atovaquone resistance (Looareesuwan et al., 1999). Atovaquone is currently used in combination with proguanil for the treatment and prophylaxis of malaria, but, because of its high price, the combination is generally limited to travellers from industrialized countries.

The efficacy of atovaquone–proguanil remained high in the four studies included in the analysis, in Cambodia, Ethiopia, Thailand and Viet Nam (Tables A1.2 and A1.3).

In previous studies, molecular analysis of recrudescent isolates showed that atovaquone resistance is associated with a single mutation at cytochrome *b*, which seems to compromise its efficacy. Mutations in this gene have been reported in Burkina Faso, Cameroon, the Comoros, Côte d'Ivoire, French Guiana, Guinea, India, Kenya, Mali, Mozambique, Nigeria, Senegal, Sierra Leone and Uganda (Patel & Kain, 2005). Mutations were also detected among patients who failed treatment due to frequent de novo mutation. In other cases, treatment failures were linked to poor absorption, which can lead to inadequate blood levels (Musset et al., 2006b; Sutherland et al., 2008). Atovaquone is a lipophilic drug, and its absorption is heavily influenced by the availability of fatty foods.

ARTEMISININ-BASED COMBINATION THERAPIES

In order to maximize the effectiveness of artemisinin and its derivatives and to protect them from the development of resistance, WHO has repeatedly recommended that they be combined with other drugs that have different mechanisms of action and longer half-lives (WHO, 2001; WHO, 2006; WHO, 2010). As artemisinin and its derivatives reduce most of the parasite biomass during their initial rapid action, an effective partner drug can usually eliminate the small number of remaining parasites. In addition, the probability of emergence of a spontaneous mutation that confers resistance to two drugs with unrelated modes of action is very low (White, 1999). While an artemisinin derivative alone must be administered over an extended period, combination therapy can be administered over a shorter period, reducing the risk for treatment failure due to poor compliance.

Five combinations are currently recommended: artemether–lumefantrine, artesunate–amodiaquine, artesunate–mefloquine, artesunate–sulfadoxine–pyrimethamine and dihydroartemisinin–piperaquine (WHO, 2010). The appropriate choice of both first- and second-line treatment should be guided by the results of therapeutic efficacy studies. With the exception of artesunate–sulfadoxine–pyrimethamine, these combinations are now available as fixed-dose treatments, which are preferable because of improved ease of use and adherence to treatment.

Artemether–lumefantrine

In anticipation of the need to protect artemisinin and its derivatives from resistance, Chinese researchers began studying ACTs in 1981 (Cui, 2009) and registered the first ACT in 1992. This was the combination of artemether and lumefantrine into a single tablet. Currently, 56 countries are using artemether–lumefantrine as first- or second-line treatment (Figure 7).

FIGURE 7. Countries in which artemether–lumefantrine is used as first- or second-line treatment (2010)

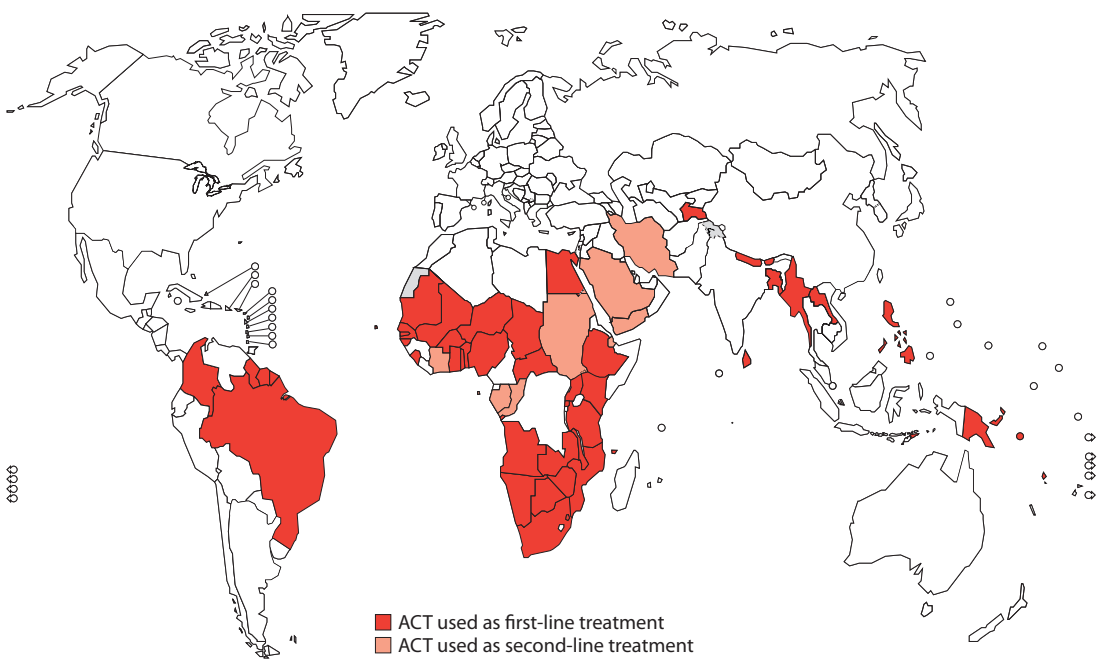
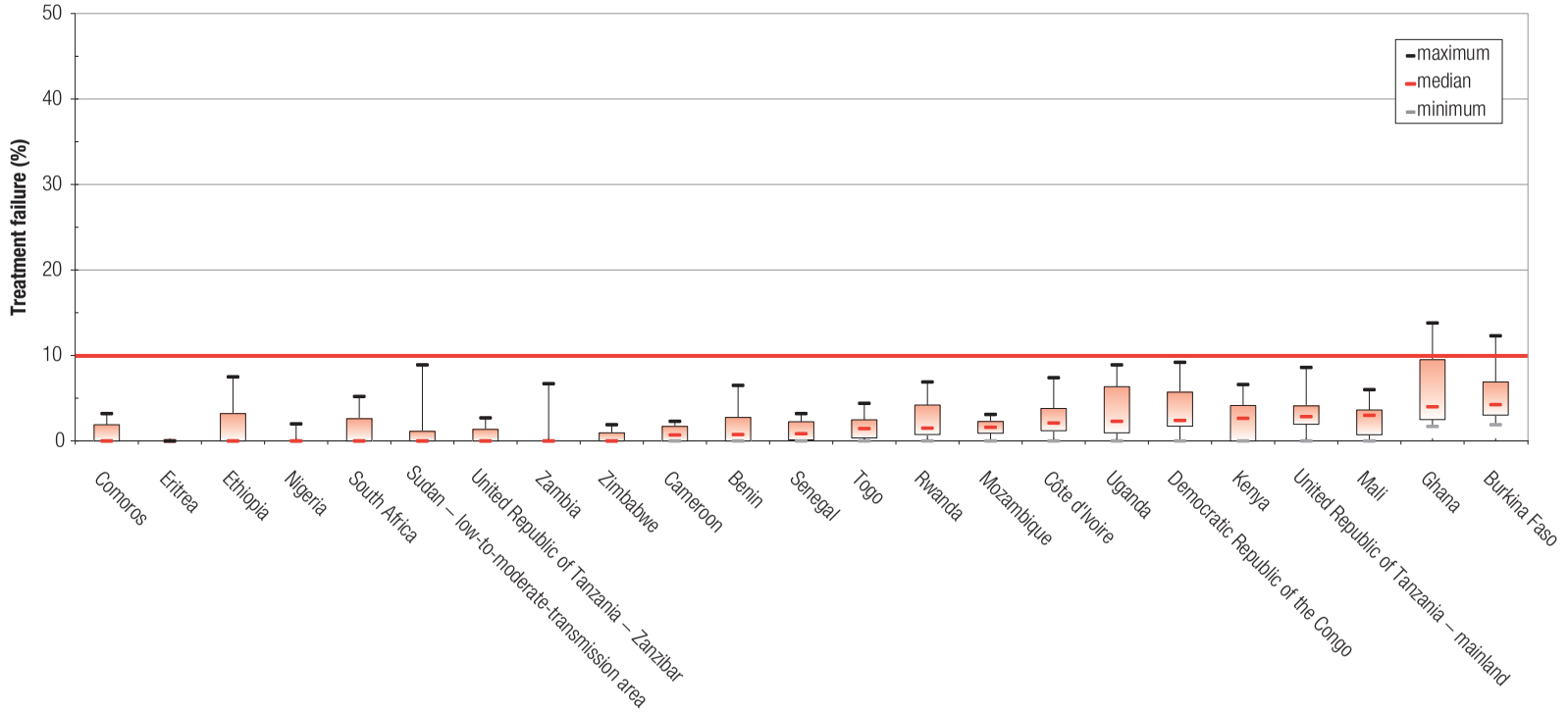


FIGURE 8. Treatment failure rates with artemether–lumefantrine in Africa (2002–2009)

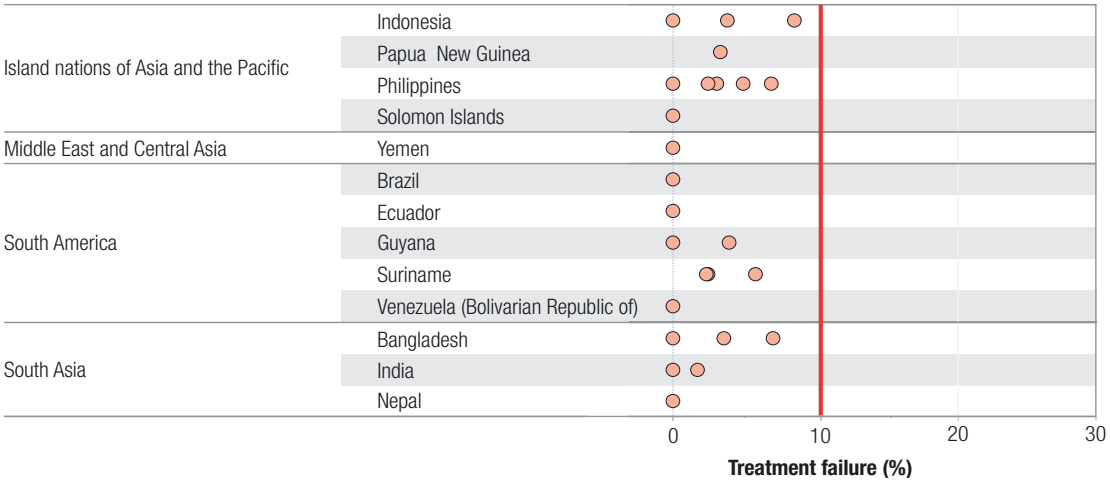


The box plots depict the following summary statistics for the efficacy studies conducted for each country and drug with a follow-up of 28 days: the minimum value (bottom whisker), the 25th percentile, the median value, the 75th percentile and the maximum value (upper whisker). Countries are sorted by median values, in ascending order. Only data for countries where three or more studies were conducted have been presented. The box plots do not illustrate variations in sample size, protocol year or study location. The red line indicates the threshold of 10% of treatment failure at which countries should initiate a change of the antimalarial medicine recommended in their national malaria treatment policy.

The analysis included 209 studies conducted in 48 countries between 2001 and 2009 (Tables A1.2 and A1.3). Over 85% of the studies in Africa found treatment failure rates of < 5%. Five studies (in the Democratic Republic of the Congo, Ghana, the Sudan – low-to-moderate-transmission area, Uganda and the United Republic of Tanzania – mainland) showed treatment failure rates of 8–10% in 2004–2008. Only two studies had treatment failure rates ≥ 10%: one in Ghana in 2006 and one in Burkina Faso in 2007 (Figure 8). While these findings appear to indicate that artemether–lumefantrine is effective in Africa, more than 85% of the studies in the database were completed in 2007 or earlier. More studies are needed to broaden understanding of the current efficacy of artemether–lumefantrine in Africa.

Studies in the island nations of Asia and the Pacific (n = 14), in South America (n = 9), in South Asia (n = 8) and in the Middle East and Central Asia (n = 1) made up 15% of the studies on artemether–lumefantrine. The treatment failure rates were < 6% in all the studies, except in Indonesia, where a 7.7% treatment failure rate was reported in 2008 (Figure 9).

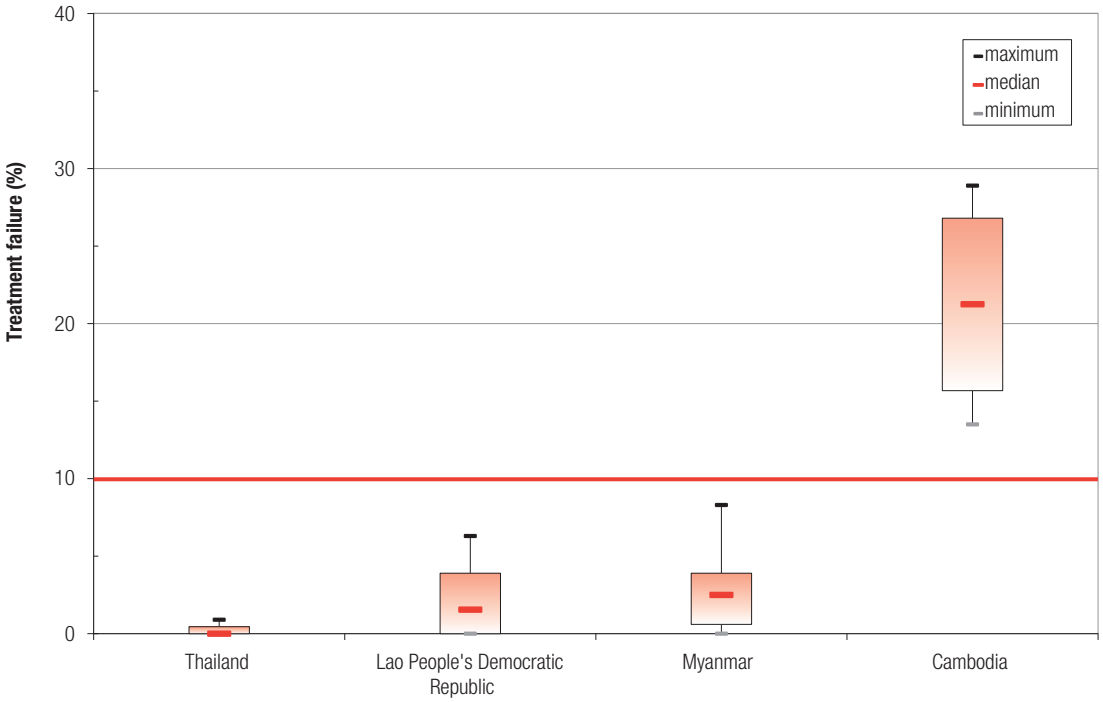
FIGURE 9. Treatment failure rates with artemether–lumefantrine by subregion (2003–2009)



The definition of each subregion is given in Annex 2. Each circle represents a study with a 28-day follow-up (except for Bangladesh where the follow-up was 42 days). The figure does not distinguish between variations in sample size, protocol year or study location. The red line indicates the threshold of 10% of treatment failure at which countries should initiate a change of the antimalarial medicine recommended in their malaria treatment policy.

The database contained 28 studies conducted in the Greater Mekong region between 2001 and 2009 (Figure 10). Over half the studies were conducted in Myanmar, where there was a median treatment failure rate of 2.5% (2004–2009). In Cambodia, studies of artemether–lumefantrine were available from 2001 to 2004. High treatment failure rates were observed in 2001 (26.1%) and 2002 (28.9%); however, in 2003, when treatment was given with fatty foods, the failure rate decreased to 13.5% (Denis et al., 2006a). Additional analyses showed that the mean plasma concentration of lumefantrine on day 7 was higher among patients with an adequate clinical and parasitological response (860 ng/ml) than among those who failed treatment (510 ng/ml). The investigators concluded that some of the treatment failures were due to low blood levels of the partner drug, lumefantrine. Owing to cross-resistance between mefloquine and lumefantrine, emergence of lumefantrine resistance could not be excluded.

FIGURE 10. Treatment failure rates with artemether–lumefantrine in the Greater Mekong subregion (2001–2009)



The box plots depict the following summary statistics for the efficacy studies conducted for each country and drug with a follow-up of 28 days: the minimum value (bottom whisker), the 25th percentile, the median value, the 75th percentile and the maximum value (upper whisker). Countries are sorted by median values, in ascending order. Only data for countries where three or more studies were conducted have been presented. The box plots do not illustrate variations in sample size, protocol year or study location. The red line indicates the threshold of 10% of treatment failure at which countries should initiate a change of the antimalarial medicine recommended in their national malaria treatment policy.

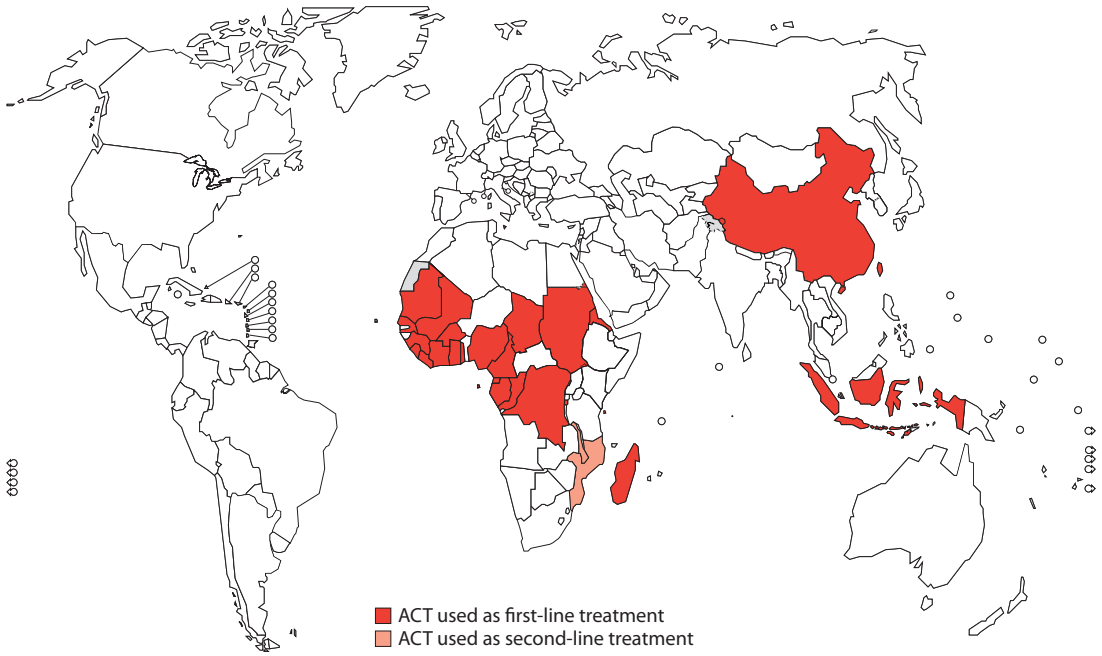
The efficacy of artemether–lumefantrine combination is strongly influenced by the wide variation in the pharmacokinetics of lumefantrine among individuals. As its absorption is enhanced by concomitant intake of fatty foods (Ezzet, Mull & Karbwang, 1998), treatment failures with this combination might be due to insufficient absorption of lumefantrine. The main determinant of the efficacy of the combination is the area under the curve of the plasma concentration of lumefantrine, or its surrogate, the plasma concentration of lumefantrine on day 7 (White, van Vugt & Ezzet, 1999; Price et al., 2006).

Artemether–lumefantrine remains highly effective in most parts of the world, with the exception of Cambodia. Although no time trends have been observed in any of subregions, continuous monitoring is necessary. Artemether–lumefantrine was reported to select for the wild-type *Pfmdr1* Asn86 allele in recurrent infections, which could be a marker of reduced susceptibility to lumefantrine. In order to ensure early detection of lumefantrine resistance, therapeutic efficacy studies should be complemented with measurement of the blood concentration of lumefantrine on day 7 and with studies of *Pfmdr1* polymorphisms.

Artesunate–amodiaquine

Amodiaquine was combined with artesunate in clinical trials conducted in Africa (Adjuik et al., 2004). Artesunate–amodiaquine was first available as a co-blister and is now also available as a fixed-dose combination. Currently, 27 countries (25 in Africa) are using artesunate–amodiaquine as first- or second-line treatment (Figure 11).

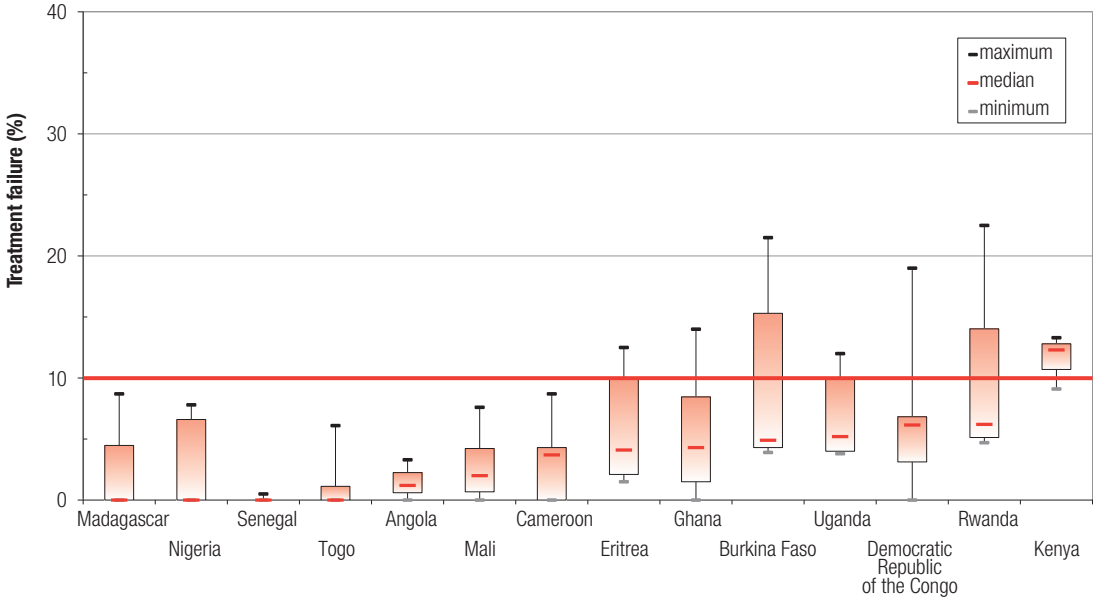
FIGURE 11. Countries in which artesunate–amodiaquine is the first- or second-line treatment (2010)



Artesunate–amodiaquine is the first-line drug in the high-transmission area of the Sudan.

The analysis included 137 studies conducted in 33 countries between 2000 and 2009 (Tables A1.2 and A1.3). The efficacy of artesunate–amodiaquine is heterogeneous in Africa, probably due to pre-existing amodiaquine resistance (Figure 12) (Zwang et al., 2009a).

FIGURE 12. Treatment failure rates with artesunate–amodiaquine in selected in selected countries in Africa (2002–2009)



The box plots depict the following summary statistics for the efficacy studies conducted for each country and drug with a follow-up of 28 days: the minimum value (bottom whisker), the 25th percentile, the median value, the 75th percentile and the maximum value (upper whisker). Countries are sorted by median values, in ascending order. Only data for countries where three or more studies were conducted have been presented. The box plots do not illustrate variations in sample size, protocol year or study location. The red line indicates the threshold of 10% of treatment failure at which countries should initiate a change of the antimalarial medicine recommended in their national malaria treatment policy.

Of the 23 African countries that have adopted artesunate–amodiaquine as first-line treatment, six (Burkina Faso, the Democratic Republic of the Congo, Eritrea, Gabon, Ghana and Sierra Leone) have reported a treatment failure rate ≥ 10% in at least one study after 28-day follow-up. The rate was unexpectedly low in Viet Nam. In Indonesia, where artesunate–amodiaquine is the first-line treatment, four of eight studies showed a treatment failure rate ≥ 10% (Figure 13); patients in these studies were treated with either 25 or 30 mg/kg body weight.

FIGURE 13. Treatment failure rates with artesunate–amodiaquine by subregion (2000–2007)

The definition of each subregion is given in Annex 2. Each circle represents a study with a 28-day follow-up (except in Afghanistan where the follow-up was 42 days). The figure does not distinguish between variations in sample size, protocol year or study location. The red line indicates the threshold of 10% of treatment failure at which countries should initiate a change of the antimalarial medicine recommended in their malaria treatment policy.

No significant changes were observed over time in the treatment failure rates in any of the subregions, but high rates were reported in Africa and in Indonesia. Countries in which artesunate–amodiaquine is used as first-line treatment should continue to monitor the failure rates to ensure that their antimalarial treatment policy is in line with efficacy data, especially if the reported treatment failure rates are ≥ 10%.

Artesunate–mefloquine

The artesunate–mefloquine combination was introduced after the spread of resistance to mefloquine in Thailand. It was first available as a co-blister and is now also available as a fixed-dose combination. Currently, eight countries are using artesunate–mefloquine as first- or second-line treatment (Figure 14).

The analysis included 80 studies conducted in 17 countries between 2000 and 2010 (Tables A1.2 and A1.3) (Figure 15). After a dose of 25 mg/kg body weight of mefloquine and 12 mg/kg body weight of artesunate over 3 days, the failure rate at 28 days was < 10% in all studies except those in Cambodia and Thailand (Figure 16).

FIGURE 14. Countries in which artesunate–mefloquine is used as first- or second-line treatment (2010)

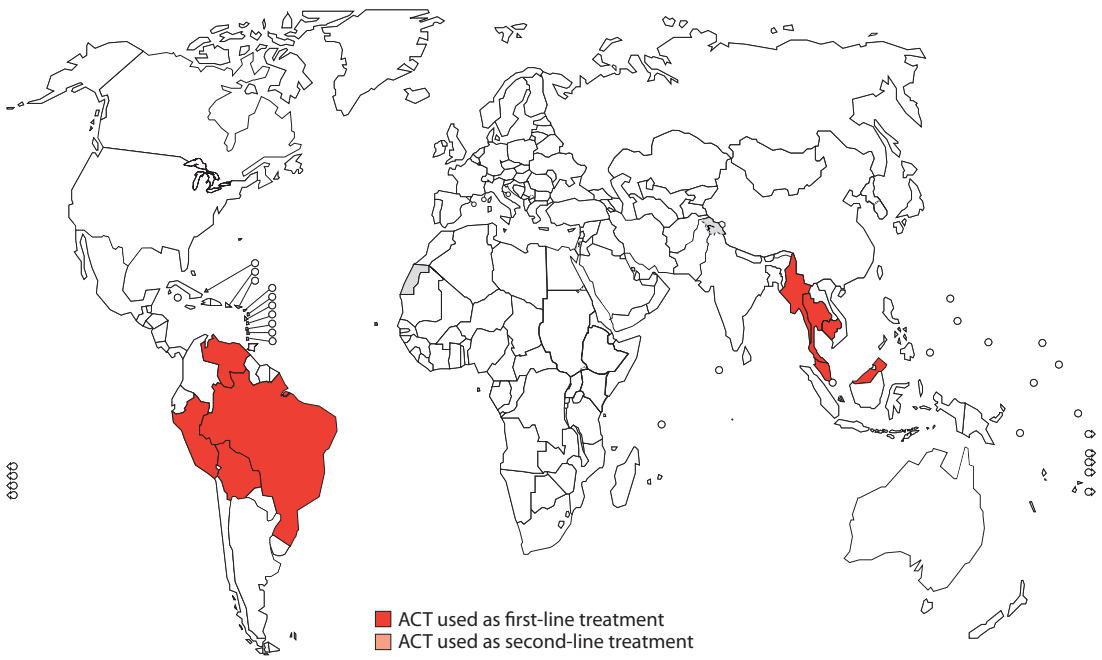
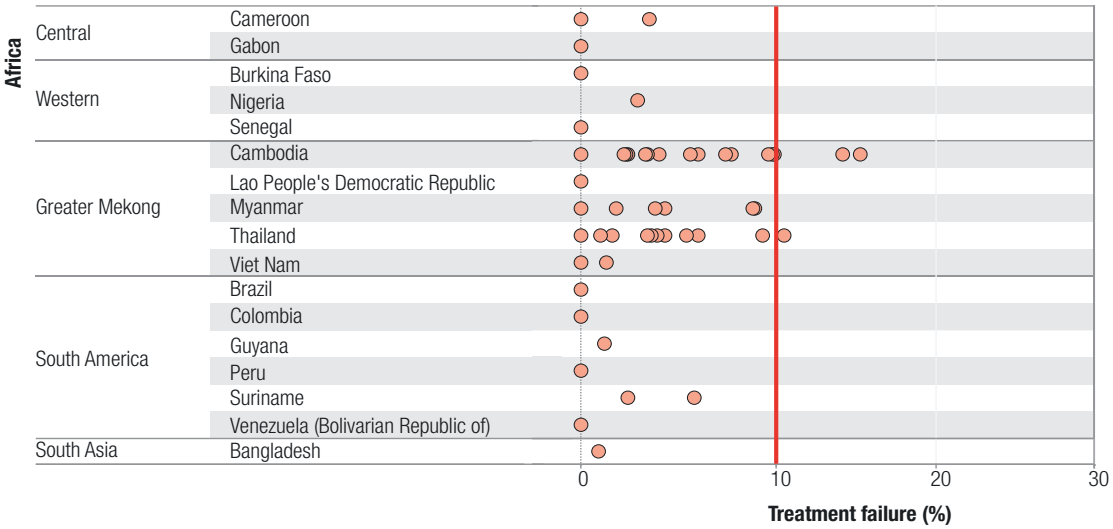
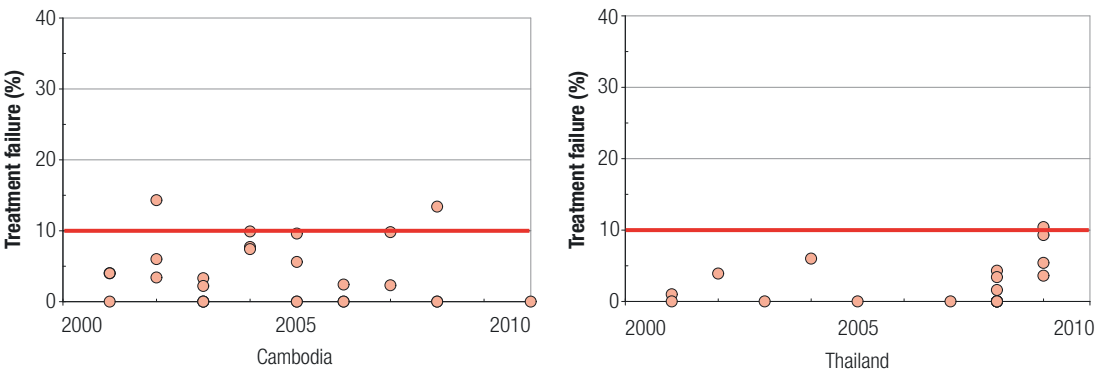


FIGURE 15. Treatment failure rates with artesunate–mefloquine by subregion (2000–2010)



The definition of each subregion is given in Annex 2. Each circle represents a study with a 28-day follow-up (except in Suriname where the follow-up was 35 days). The figure does not distinguish between variations in sample size, protocol year or study location. The red line indicates the threshold of 10% of treatment failure at which countries should initiate a change of the antimalarial medicine recommended in their malaria treatment policy.

FIGURE 16. Treatment failure rates with artesunate–mefloquine in Cambodia and Thailand (2001–2010)



Each circle represents a study with a 28-day follow-up. The figure does not distinguish between variations in sample size, protocol year or study location. The red line indicates the threshold of 10% of treatment failure at which countries should initiate a change of the antimalarial medicine recommended in their malaria treatment policy.

Nine studies showing treatment failure rates of 8.8–14% were reported between 2002 and 2010 in Cambodia, Myanmar and Thailand. Treatment failure rates ≥ 10% have been reported in Cambodia since 2002, whereas in Thailand the rate of treatment failure has increased over the past 10 years with one study in 2009 showing a treatment failure > 10%. The failure rates after 42 days of follow-up have been reported as high as 20% and 12% in Cambodia and in Thailand, respectively.

In Pailin Province, the rate of treatment failure with artesunate–mefloquine decreased from 9.9–14.3% in 2002–2004 to 0–5% in 2007–2008. The decrease was associated with implementation of rapid diagnostic tests and replacement of artesunate–mefloquine by dihydroartemisinin–piperaquine at community level. The dramatic re-emergence of mefloquine-sensitive parasites in Pailin was confirmed by molecular analysis, which showed deamplification of *Pfmdr1* copy numbers between 2005 and 2007 (Imwong et al.,

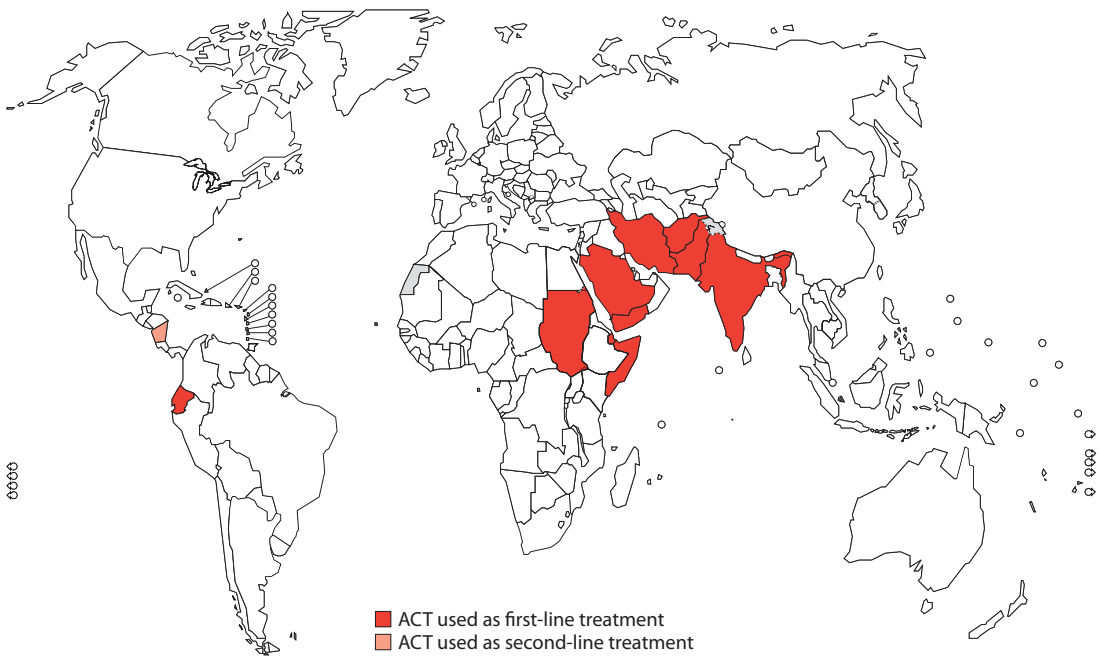
2010). These findings suggest that the high treatment failure rates with artesunate–mefloquine observed in 2002–2004 were due to mefloquine resistance. In Africa and the Americas, artesunate–mefloquine remains highly effective.

Because of the long half-life of mefloquine, the efficacy of artesunate-mefloquine must be monitored for at least 42 days. Artesunate–mefloquine is failing mainly in areas where mefloquine resistance is highly prevalent. Regardless of whether these failures are due only to mefloquine resistance or to resistance to both mefloquine and artesunate, countries in the Greater Mekong subregion should continue to monitor the efficacy of this combination carefully and review their treatment policies accordingly. Further spread of mefloquine resistance in areas where there is artemisinin resistance and where artesunate–mefloquine is used as first-line treatment could jeopardize efforts to contain artemisinin resistance.

Artesunate–sulfadoxine–pyrimethamine

Artesunate–sulfadoxine–pyrimethamine is the only ACT recommended by WHO that is not available as a fixed-dose combination. Currently, 11 countries are using artesunate–sulfadoxine–pyrimethamine as first- or second-line treatment (Figure 17).

FIGURE 17. Countries in which artesunate–sulfadoxine–pyrimethamine is used as first- or second-line treatment (2010)

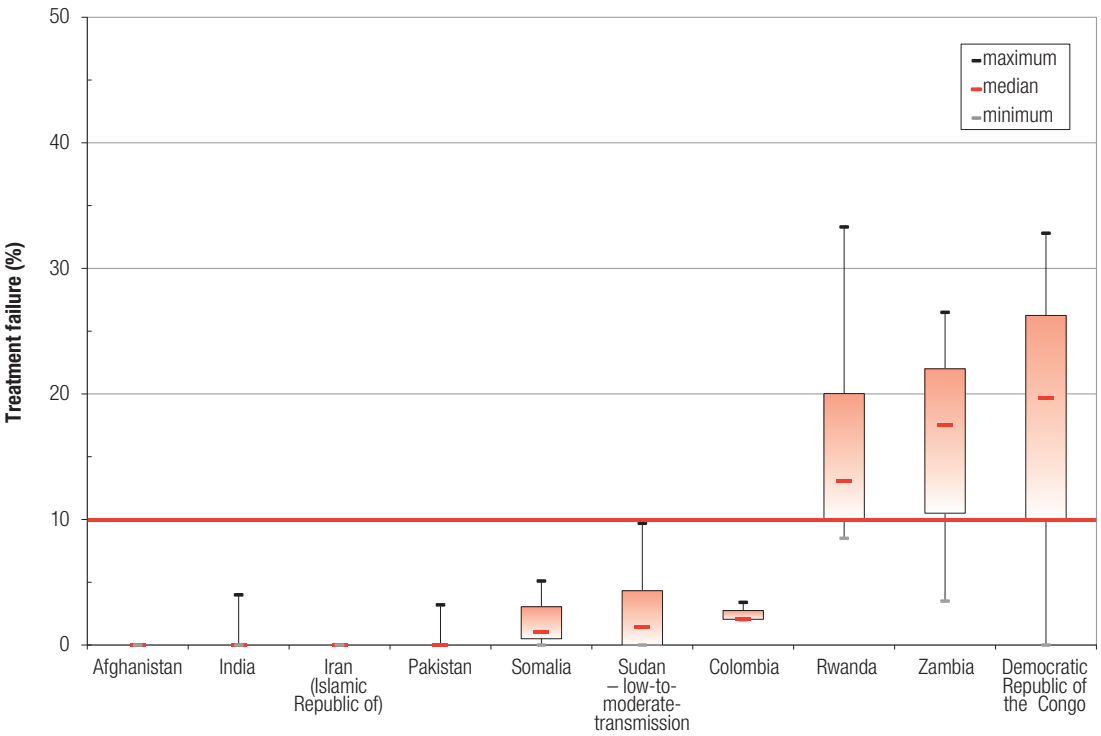


Artesunate–sulfadoxine–pyrimethamine is the first-line drug in the low-to-moderate-transmission area of the Sudan.

The analysis included 77 studies conducted in 28 countries between 2000 and 2008 (Tables A1.2 and A1.3). Artesunate–sulfadoxine–pyrimethamine treatment failure rates remained low in studies conducted in the Americas (Colombia, Ecuador and coastal areas of Peru), the Middle East and Central Asia (Afghanistan, the Islamic Republic of Iran, Pakistan, Tajikistan and Yemen), South Asia (India and Sri Lanka) and eastern Africa (Somalia and the Sudan) and particularly in the countries in which artesunate–pyrimethamine–sulfadoxine is used as first-line treatment, where the median treatment failure rate was 0–1.5%. The high

clinical efficacy of this combination may be due partially to the rarity of the *Pfdhfr* and *Pfdhps* quintuple mutant (Zakeri et al., 2010). In contrast, high failure rates of this combination have been observed in several African countries where sulfadoxine–pyrimethamine resistance is high (Figure 18).

FIGURE 18. Treatment failure rates with artesunate–sulfadoxine–pyrimethamine in selected countries (2001–2008)



The box plots depict the following summary statistics for the efficacy studies conducted for each country and drug with a follow-up of 28 days: the minimum value (bottom whisker), the 25th percentile, the median value, the 75th percentile and the maximum value (upper whisker). Countries are sorted by median values, in ascending order. Only data for countries where three or more studies were conducted have been presented. The box plots do not illustrate variations in sample size, protocol year or study location. The red line indicates the threshold of 10% of treatment failure at which countries should initiate a change of the antimalarial medicine recommended in their national malaria treatment policy.

Although artesunate–sulfadoxine–pyrimethamine is currently highly effective in countries that have adopted this ACT as first-line treatment, continuous monitoring is needed. Sulfadoxine–pyrimethamine resistance is present in most endemic countries, and there have been no consistent or well-documented reports that resistance decreases after it is established. The addition of molecular studies of *Pfdhfr* and *Pfdhps* to the therapeutic efficacy studies would be helpful for interpreting data and trends.

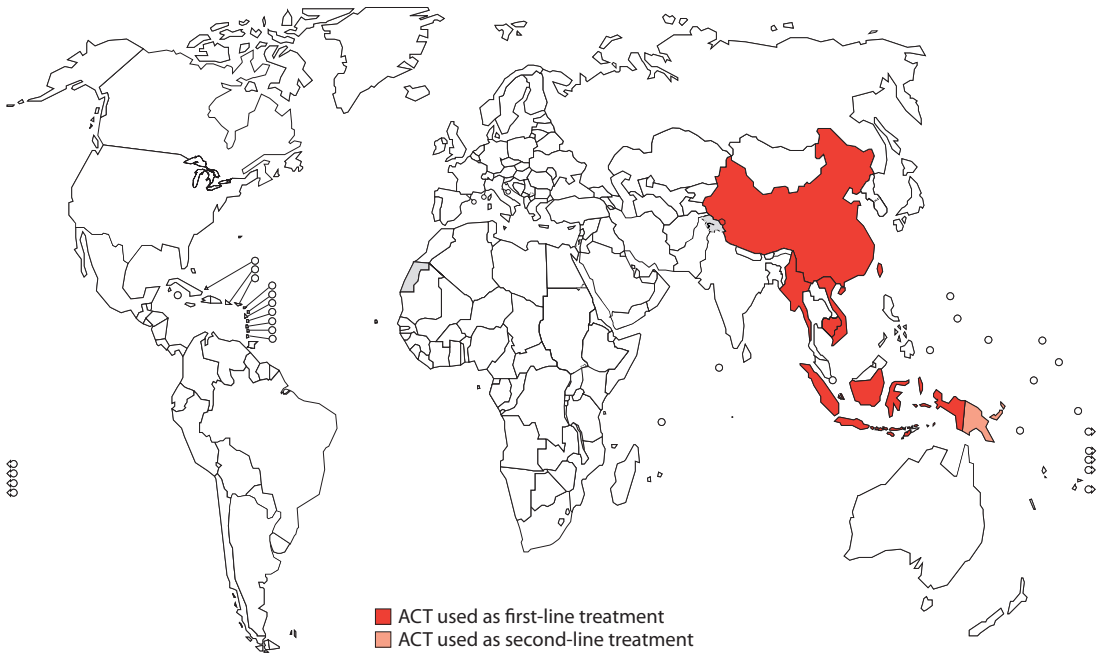
Artesunate–sulfalene–pyrimethamine

Sulfalene is a sulfonamide that is available in a fixed-dose combination with artesunate and pyrimethamine. In the 2010 *Guidelines for the treatment of malaria* (WHO, 2010), sulfalene is considered an alternative to sulfadoxine. The two are chemically related but have slightly different biological properties, with regard to their half-lives and the fraction bound to proteins. All 10 studies conducted in Africa showed a consistently high efficacy of the artesunate–sulfalene–pyrimethamine combination.

Dihydroartemisinin–piperaquine

Piperaquine is a bisquinoline developed independently in the 1960s by Chinese investigators and the French pharmaceutical company Rhone Poulenc. It was used widely for the treatment and prevention of malaria in China in the 1980s; however, resistance to piperaquine eventually emerged, which led to its use in combination therapy (Davis et al., 2005). The most widely studied combination is dihydroartemisinin–piperaquine, which is now one of the five ACTs recommended by WHO. The countries in which this combination is used are shown in Figure 19.

FIGURE 19. Countries in which dihydroartemisinin–piperaquine is used as first- or second-line treatment (2010)



Dihydroartemisinin–piperaquine is the first-line treatment in zone 1 of the artemisinin resistance containment project in Cambodia, and in West Papua and Papua provinces, Indonesia.

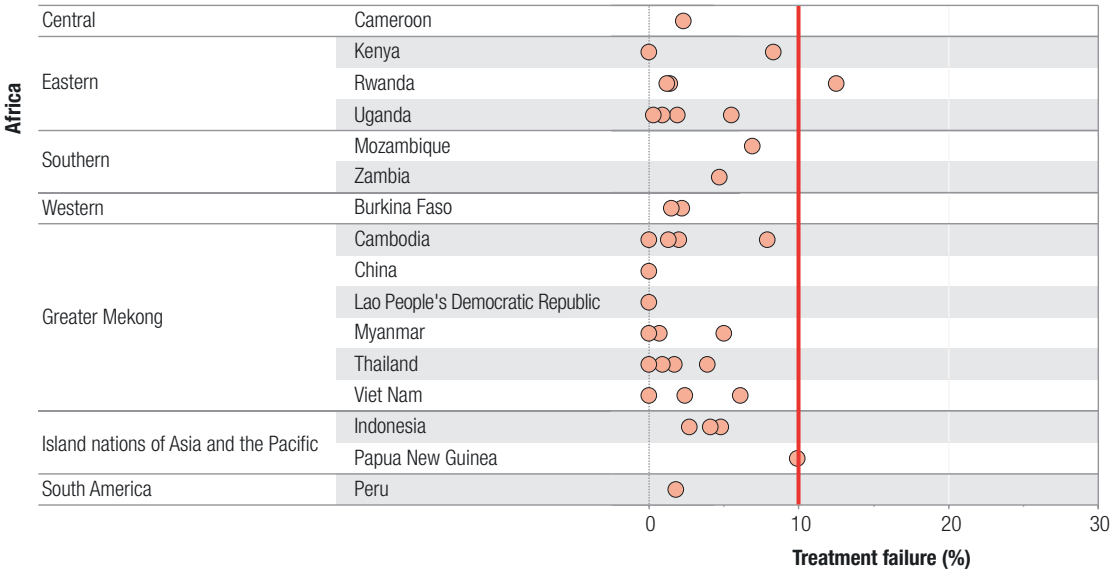
Many trials to monitor the safety and efficacy of dihydroartemisinin–piperaquine have been conducted in Africa and South-East Asia (Bassat et al., 2009; Zwang et al., 2009b; Valecha et al., 2010). Forty-nine of these studies, conducted with either three equal doses over 3 days or a double dose on the first day and single doses on days 2 and 3, in 16 countries between 2002 and 2010 were eligible for inclusion in the analysis (Tables A1.2 and A1.3). The treatment failure rates in all studies were < 10%, except in one study in Rwanda in 2004; one study in Papua New Guinea showed a 9.9% treatment failure (Figure 20). When the follow-up was extended to 42 days, treatment failure rates of 9.1–12% were reported in Burkina Faso, Cambodia, Kenya, Mozambique, Papua New Guinea and Uganda.

Several combinations of dihydroartemisinin and piperaquine have been produced and marketed: dihydroartemisinin–piperaquine–trimethoprim–primaquine, dihydroartemisinin–piperaquine–trimethoprim and dihydroartemisinin–piperaquine. A clinical trial showed, however, that the inclusion of trimethoprim does not change the treatment efficacy (Tran et al., 2004).

As observed with artemether–lumefantrine, the major determinant of the parasitological failure of dihydroartemisinin–piperaquine is the plasma concentration of piperaquine on day 7. In a study in Papua, Indonesia, patients with piperaquine levels < 30 ng/ml were more likely to have recrudescence of *P. falciparum* or *P. vivax* malaria (Price et al., 2007).

Because of the long half-life of piperaquine, the efficacy of dihydroartemisinin–piperaquine must be monitored for at least 42 days. This combination is the latest ACT recommended by WHO, although quality-assured products have not yet become available. Most of the studies that showed good efficacy were conducted in limited areas of Africa and in the Greater Mekong subregion.

FIGURE 20. Treatment failure rates with dihydroartemisinin–piperaquine by subregion (2001–2009)



The definition of each subregion is given in Annex 2. Each circle represents a study with a 28-day follow-up (except in Indonesia where the follow-up was 42 days and in Peru and Thailand where the follow-up was 63 days). The figure does not distinguish between variations in sample size, protocol year or study location. The red line indicates the threshold of 10% of treatment failure at which countries should initiate a change of the antimalarial medicine recommended in their malaria treatment policy.

Other ACT in development

Pyronaridine is a mannich base that was synthesized in China in 1970, and this medicine has been widely used alone or in combination for the treatment of uncomplicated malaria in that country (Fu & Xiao 1991; Liu et al., 2002). The analysis included eight studies of artesunate–pyronaridine conducted in Africa and Asia between 2006 and 2008, as part of phase II and phase III trials. The artesunate–pyronaridine treatment failure rates were 0–0.5% (Tables A1.2 and A1.3). This combination has been submitted for approval to stringent regulatory authorities.

OTHER COMBINATION THERAPIES

Non-ACT combinations, such as chloroquine–sulfadoxine–pyrimethamine and amodiaquine–sulfadoxine–pyrimethamine, were once proposed as alternatives to ACTs for treatment; however, with the spread of resistance to chloroquine and sulfadoxine–pyrimethamine, these combinations are no longer recommended by WHO (WHO, 2010).

CONCLUSIONS ON COMBINATION THERAPIES

ACTs remain the most effective treatment for uncomplicated *P. falciparum* malaria. Artemisinin derivatives are very effective in rapidly reducing the parasite biomass, and, when combined with an effective partner medicine, they are likely to clear all parasites successfully. On the basis of current understanding of resistance to artemisinin and its derivatives, the delayed parasite clearance currently observed in areas of resistance to artemisinin and its derivatives does not seem to affect the overall efficacy of ACTs, provided the partner medicine remains fully effective. There are, however, many unknowns about the future efficacy of ACTs. It is not clear whether parasite clearance time will continue to increase, how resistance to artemisinin and its derivatives will evolve and how this will eventually affect the efficacy of ACTs. It is therefore extremely important that countries monitor the efficacy of their first- and second-line ACTs, so that they can change to a different ACT with a different partner medicine if the rate of treatment failure exceeds 10%. This is particularly important in countries where the failure rate of the first-line drug is approaching 10%, even if only at a limited number of sites. Monitoring should also include an evaluation of the proportion of patients who are still parasitaemic on day 3, in order to detect early signs of resistance to artemisinin and its derivatives.

Addition of a gametocytocidal medicine, such as primaquine, to an ACT might provide an added benefit. Artemisinin and its derivatives appear to have activity against younger gametocyte stages but appear inactive against mature gametocytes, a developmental stage which primaquine effectively kills or sterilizes. The two thus act synergistically (Pukrittayakamee et al., 2004). A recent study in Myanmar showed that a single dose of primaquine (0.75 mg/kg) combined with an ACT reduced *P. falciparum* gametocyte carriage substantially, and was well tolerated (Smithuis et al., 2010). Addition of a single dose of primaquine to ACT treatment for uncomplicated falciparum malaria as an antigametocyte, particularly as a component of pre-elimination or an elimination programme, is therefore recommended (WHO, 2010).

3.2 *Plasmodium vivax*

The following section is a compilation of data from the literature and data provided by countries. Resistance of *P. vivax* is limited to chloroquine, sulfadoxine–pyrimethamine and primaquine, and few studies have been conducted on other drugs.

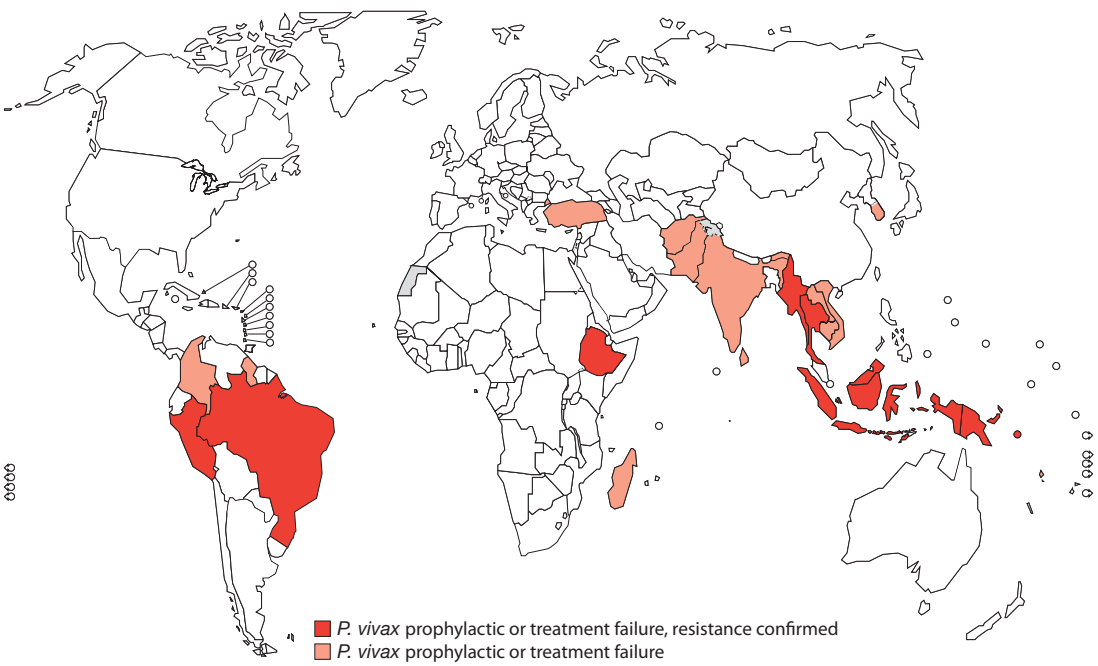
CHLOROQUINE

Chloroquine remains the first-line treatment for vivax malaria in all countries except Indonesia, the Solomon Islands and Vanuatu, where ACTs are used. Concurrent primaquine therapy probably improves the activity of chloroquine against resistant blood-stage parasites (Pukrittayakamee et al., 1994; Baird et al., 1995).

Resistance to chloroquine was first reported in the late 1980s in Indonesia and Papua New Guinea. Treatment failure in the presence of parasites, despite a blood concentration > 100 ng/ml, has been suggested to be a good marker of resistance of *P. vivax* to chloroquine (Baird et al., 1997), although this estimate requires further validation. Concentrations of chloroquine of 15 ng/ml in plasma and 30 ng/ml in serum have been considered to be the minimum effective concentrations for suppressing *P. vivax*; the concentrations in whole blood are usually several times higher than those in plasma or serum. In addition, the metabolite monodesethylchloroquine can also act against *P. vivax*, as it does against *P. falciparum*. It can be assumed that chloroquine-sensitive *P. vivax* will be suppressed by whole-blood concentrations of 70–90 ng/ml of chloroquine and its metabolite.

Prophylactic and treatment failure and confirmed resistance of *P. vivax* are shown in Figure 21. Treatment failure on or before day 28 or prophylactic failure have been observed in Afghanistan, Brazil, Cambodia, Colombia, Guyana, Ethiopia, India, Indonesia, Madagascar, Malaysia (Borneo), Myanmar, Pakistan, Papua New Guinea, Peru, the Republic of Korea (after treatment with hydroxychloroquine), the Solomon Islands, Sri Lanka, Thailand, Turkey, Vanuatu and Viet Nam (Baird, 2009). In only some of these studies, however, was the chloroquine drug concentration measured in order to confirm the presence of chloroquine resistance. At least one true case of chloroquine resistance (with whole blood concentrations of chloroquine plus desethylchloroquine > 100 ng/ml on the day of failure) has been confirmed in Brazil, Ethiopia, Indonesia, Malaysia (Borneo), Myanmar, Papua New Guinea, Peru, the Solomon Islands and Thailand.

FIGURE 21. Prophylactic and treatment failure and confirmed resistance of *P. vivax* to chloroquine



Parasites carrying the Tyr976Phe mutation of *Pvmdr1* showed reduced susceptibility to chloroquine in vitro when compared with wild-type parasites from Indonesia and Thailand; however, this marker was not found to be useful for monitoring chloroquine resistance in Madagascar (Barnadas et al., 2008; Suwanarusk et al., 2008).

MEFLOQUINE

Mefloquine is highly effective against chloroquine-resistant vivax malaria (Maguire et al., 2006). After widespread use of this drug in the Greater Mekong subregion, an increased prevalence of the vivax parasite with *Pvmdr1* gene amplification was seen. In vitro, the Tyr976Phe *Pvmdr1* mutation has been associated with increased susceptibility to mefloquine and artesunate. Further studies to define the clinical correlates of Tyr976Phe are needed (Imwong et al., 2008; Suwanarusk et al., 2008).

SULFADOXINE-PYRIMETHAMINE

The molecular basis of pyrimethamine resistance has been extensively studied. Key mutations at codons 57, 58, 61 and 117 of the *Pvdhfr* gene have been associated with clinical failure. Several clinical studies have indicated that sulfadoxine–pyrimethamine remains effective for patients infected with wild-type *P. vivax*. Unfortunately, the key mutations have been found in many malaria-endemic countries, potentially jeopardizing the efficacy of this medicine (Imwong et al., 2005; Hawkins et al., 2007; Lu et al., 2010).

Until recently, the molecular basis of *P. vivax* resistance to sulfadoxine was poorly documented. *P. vivax* shows some degree of 'innate resistance' to sulfadoxine, which can be enhanced by drug selection. The *Pvdhps* 585 wild-type residue is responsible for this 'innate resistance', and mutations at codon 383 and 553 could be responsible for increased resistance (Korsinczky et al., 2004).

PRIMAQUINE

Primaquine is higly active against hypnozoites of the relapsing parasites *P. vivax* and *P. ovale*. The anti-relapse effect of primaquine is a function of the total dose rather than the duration of treatment. Geographical variations in the sensitivity of hypnozoites of *P. vivax* to primaquine have led to a recommendation for different regimens. Although there have been several reports of *P. vivax* resistance to primaquine, the data must be interpreted with caution because of many confounding factors, such as geographical variations in relapse patterns, unsupervised therapy, parasite tolerance, the risk for reinfection and the difficulty of finding a valid control group (Goller et al., 2007; Baird, 2009; WHO, 2009a).

ARTEMISININ-BASED COMBINATION THERAPY

Clinical studies have shown that ACTs can clear *P. vivax* infections more quickly than chloroquine. High cure rates with ACTs have been observed in Afghanistan, Indonesia and Thailand, but not in Papua New Guinea, where the failure rates with artemether–lumefantrine, artesunate–sulfadoxine–pyrimethamine and dihydroartemisinin–piperaquine were > 10% (Tjitra et al., 2002; Hasugian et al., 2007; Kolaczinski et al., 2007; Krudsood et al., 2007; Ratcliff et al., 2007; Karunajeewa et al., 2008; Awab et al., 2010). Whereas in falciparum malaria the early action of artemisinin and its derivatives can limit the development of gametocytes, these drugs have a less significant effect on the transmission of *P. vivax*, as its gametocytes may exist even before treatment is administered (Douglas et al., 2010).

ACTs are recommended for the treatment of chloroquine-resistant *P. vivax* and where ACTs have been adopted as first-line treatment for *P. falciparum*. In view of the resistance of *P. vivax* to sulfadoxine–pyrimethamine, artesunate–sulfadoxine–pyrimethamine may not be the most appropriate ACT for treating vivax malaria.

Countries are encouraged to monitor the efficacy of chloroquine and ACTs against *P. vivax*. In areas where both falciparum and vivax are prevalent, existing sentinel sites can be used to monitor therapeutic efficacy against falciparum and vivax malaria, both simultaneously and independently. The addition of chloroquine pharmacokinetics to clinical trials will allow better definition of chloroquine resistance. The data collected by countries will be added to a WHO database on *P. vivax* that will be established in 2011, which will improve understanding of the trends in and the extent of *P. vivax* resistance to chloroquine and will help determine which ACTs are the most appropriate for treating vivax malaria.

3.3 Other species

The resistance of *P. ovale* and *P. malariae* to antimalarials is not well characterized, and these infections are considered to be generally sensitive to chloroquine. Only one study in Indonesia reported resistance of *P. malariae* to chloroquine (Maguire et al., 2002). Chloroquine remains fully effective against *P. malariae* in Madagascar and against *P. knowlesi* in Malaysia (Barnadas et al., 2007; Daneshvar et al., 2010).

4. Resistance to artemisinin on the Cambodia–Thailand border

The Cambodia–Thailand border has historically been the initial focus of resistance to antimalarial treatment in the Greater Mekong subregion. The region was the first to show signs of *P. falciparum* resistance to chloroquine, sulfadoxine–pyrimethamine and mefloquine. Resistance to the first two drugs eventually spread across the Greater Mekong through India to Africa, steadily rendering treatment ineffective in most malaria-endemic countries. This section describes how early detection of reduced susceptibility of *P. falciparum* to artemisinins, by regular surveillance of antimalarial drug efficacy, led to subsequent confirmation and a coordinated international response to contain the spread of resistance. The timeline of events is presented in Figure 22.

4.1 Monitoring antimalarial drug efficacy in the Greater Mekong subregion

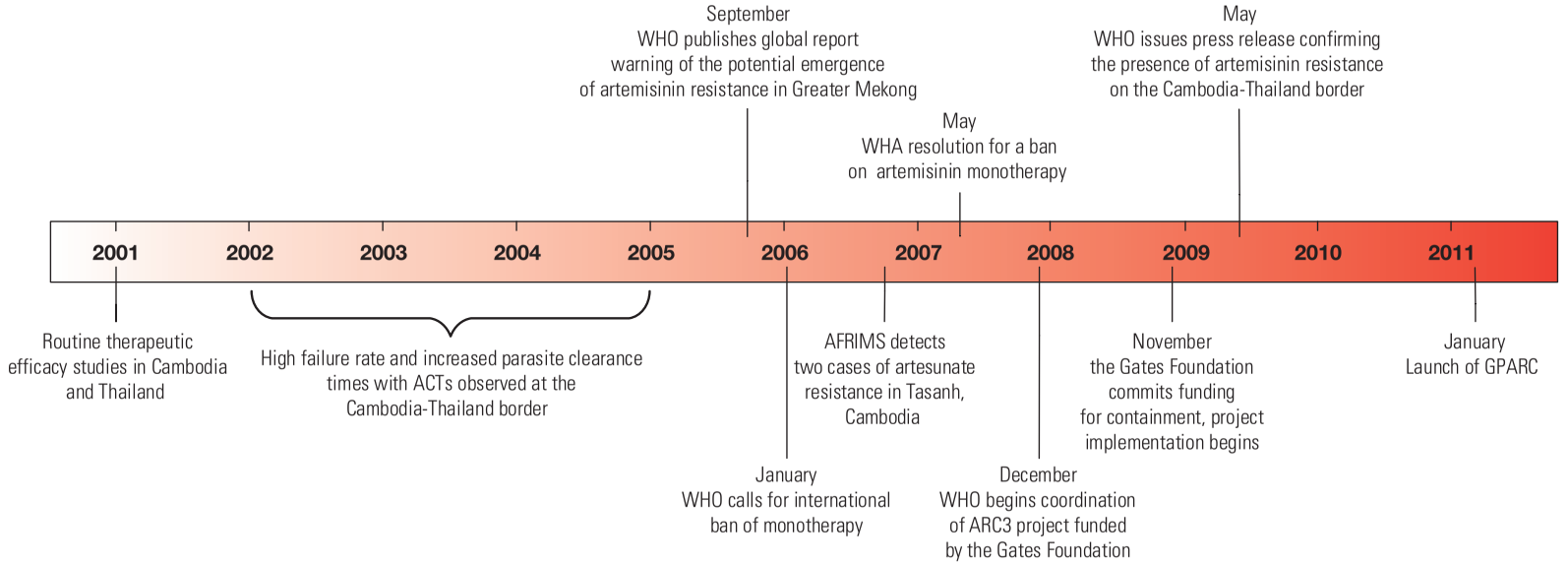
CAMBODIA

The National Centre for Parasitology, Entomology and Malaria Control in Cambodia has been monitoring the efficacy of drugs against *P. falciparum* regularly since 1991. Mefloquine monotherapy was the recommended treatment from 1993 to 2000; however, after resistance was found throughout the country, the first-line treatment policy was changed to a 3-day course of artesunate plus mefloquine in 2000. Cambodia was the first country to adopt an ACT as first-line treatment in their national policy.

WHO has supported the National Centre for Parasitology, Entomology and Malaria Control in Cambodia in conducting 28- and 42-day therapeutic efficacy studies of artesunate–mefloquine since 2001. In studies conducted between 2001 and 2004, the percentage of patients with treatment failure was < 5% at most sites. Investigators were, however, concerned about Pailin Province, where, in 2002, 14.3% of patients had late treatment failure at day 28. In 2004, a high percentage of treatment failures (9.9%) was confirmed (Denis et al., 2006b). Given the history of mefloquine resistance in the region, it was initially considered that the treatment failures were due to decreased efficacy of the partner drug, mefloquine. It was subsequently found, however, that about 10% of patients in the study had not cleared parasites by day 3 (Alker et al., 2007), and the parasite clearance times were longer than those previously reported for artesunate–mefloquine (Price et al., 1997). At about the same time, between 2001 and 2003, the efficacy of artemether–lumefantrine was being studied in Battambang Province, northwestern Cambodia (Denis et al., 2006a). Apart from a high failure rate, analyses showed that 13.8–32.7% of patients were presenting with parasites at day 3.

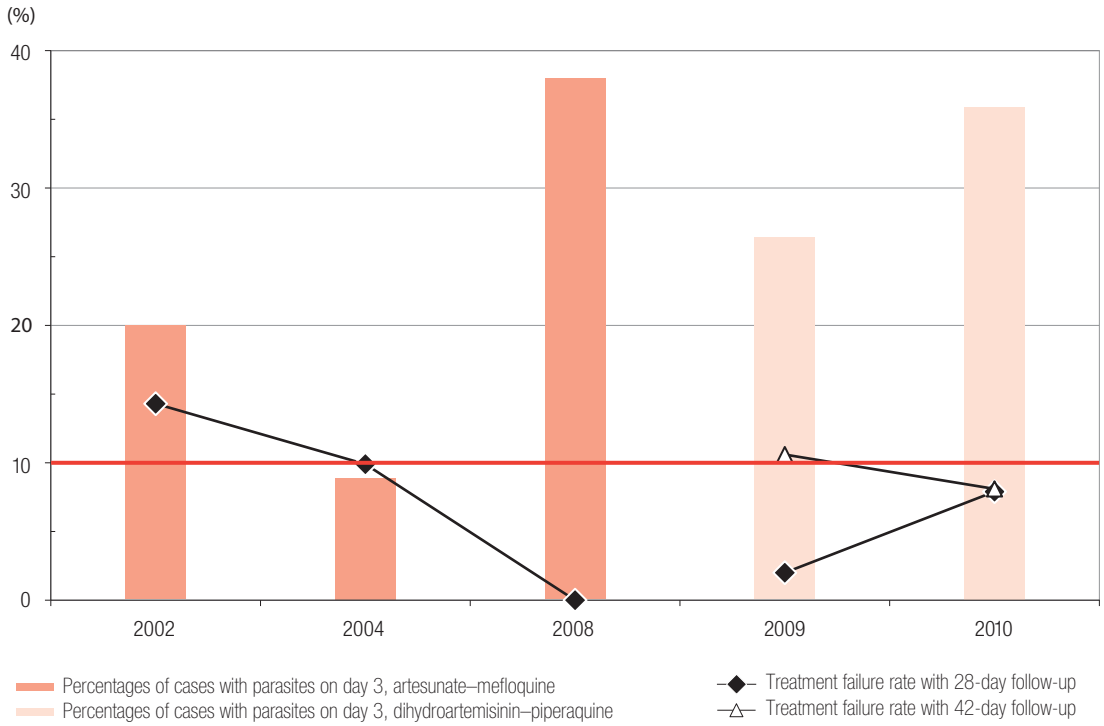
In Pailin, the treatment failure rate of artesunate–mefloquine decreased from 9.9–14.3% in 2002–2004 to 0-5% in 2007–2008, which was associated with implementation of rapid diagnostic tests and the replacement of artesunate–mefloquine by dihydroartemisinin–piperaquine at community level. These clinical findings were confirmed in a study in which deamplification of *Pfmdr1* copy numbers was demonstrated with molecular markers between 2005 and 2007 (Imwong et al., 2010). These findings appear to indicate that the high failure rate observed with artesunate–mefloquine in 2002 was due to mefloquine resistance. The delayed parasite clearance time now being observed appears to be linked to the artemisinin component and is not predictive of treatment failure with ACTs at this early stage of artemisinin resistance (Figure 23).

FIGURE 22. Schematic representation of events in the emergence of and response to artemisinin resistance (2001–2011)



AFRIMS, Armed Forces Research Institute of Medical Sciences; ARC3, artemisinin resistance project: pilot studies to confirm, characterize and plan for containment; the Gates Foundation, Bill & Melinda Gates Foundation; GPARC, Global Plan for Artemisinin Resistance Containment; WHA, World Health Assembly.

FIGURE 23. Percentage of cases with parasites on day 3 and treatment failure rate after treatment with artemisinin-based combination therapy, Pailin Province, Cambodia (2002–2010)



THAILAND

In Thailand, where the malaria incidence has been drastically reduced with a highly developed system for malaria control, an extensive network of services and facilities offer free diagnosis and treatment to both Thai and foreign nationals. Nevertheless, the emergence of drug resistance remains a serious threat to malaria control, and drug efficacy has been monitored regularly since the early 1980s, the results being used to update treatment policy. In 1995, after the emergence of *P. falciparum* resistance to mefloquine monotherapy in some provinces, the treatment policy in those provinces was changed to a 2-day artesunate–mefloquine combination (Vijaykadga et al., 2006). Mefloquine was used at a dose of 15–25 mg/kg body weight, according to the level of mefloquine resistance. The artesunate–mefloquine combination was effective when it was introduced, despite pre-existing resistance to mefloquine (Nosten et al., 2000); however, declining efficacy of the combination was later observed in Trat Province on the Cambodian border, when the adequate clinical and parasitological response decreased from 93% in 1997 and 92.5% in 1998 to 84.6% in 2002 (Rojanawatsirivej et al., 2003). Over the same period, a significant increase in the *Pfmdr1* copy number (from 17.1 to 61.9%; $p < 0.001$) was observed among parasite strains collected in Trat and Chantaburi provinces (Mungthin et al., 2010). By 2005, the 2-day artesunate–mefloquine combination was being used in all provinces. In 2008, the treatment policy for the entire country was harmonized to artesunate (3 days) mefloquine (2 days) and primaquine (1 day) (Congpuong et al., 2010).

Subsequent analysis of surveillance data on artesunate–mefloquine efficacy in Trat Province in 2003–2007 showed a corresponding increase in parasite clearance time (S. Vijaykadga, unpublished data). Artesunate–

mefloquine efficacy has been surveyed routinely since 1995 in Tak Province at the Myanmar–Thailand border by the Shoklo Malaria Research Unit. The treatment outcomes of patients who received the 3-day regimen of mefloquine and artesunate in northwestern Thailand between 1995 and 2007 have been analysed for trends over time (Carrara et al., 2009). The study sites included migrant clinics and camps for displaced persons on the Myanmar border. The analysis demonstrated subtle changes in treatment outcomes over time. For example, on average, 95.5% of patients treated between 1995 and 2001 were free of parasites after 48 h, and this percentage decreased to 78.1% between 2001 and 2007 ($p < 0.001$). Decreases in the percentages of patients able to clear parasites after 72 h were also observed: in 2001, 99.8% patients had cleared their parasitaemia by day 3, while in 2005 this fell to 95.9% ($p < 0.001$). A similar trend was reported in the neighbouring province of Kanchanaburi, where the percentage of patients able to clear parasites at day 3 decreased from 100% in 2005 to less than 80% in 2009 (Congpuong et al., 2010).

The increases in the proportions of patients parasitaemic at day 3 observed in both Cambodia and Thailand indicate that the efficacy of artesunate is diminishing. The more subtle increase in parasite clearance time on the Myanmar–Thailand could also be the result of waning immunity, as transmission of falciparum malaria dropped substantially over the same period.

4.2 Evidence of resistance of *P. falciparum* to artemisinins and early response

All signs of reduced sensitivity to artemisinins in the Greater Mekong subregion are of paramount concern to WHO and malaria control partners, given that resistance to most other antimalarials emerged in this region and that there are no other effective treatments currently available. In its report, *Susceptibility of* Plasmodium falciparum *to antimalarial drugs* (WHO, 2005), WHO recommended enhanced monitoring due to the changes in *P. falciparum* sensitivity to artemisinins in the Greater Mekong subregion, as the medicines became more widely used.

IN VITRO STUDIES

At the time of the 2005 WHO report, some evidence from in vitro monitoring over time in China and Viet Nam indicated increased IC_{50}, IC_{90} or IC_{99} values for artemisinins (Huong et al., 2001; Yang et al., 2003). Subsequently, in a comparison of samples from Bangladesh, western Cambodia and western and eastern Thailand, decreasing in vitro susceptibility was observed from west to east (Noedl, Socheat & Satimai, 2009). In a separate study in Cambodia, the highest IC_{50} was reported in the western part of the country (Lim et al., 2010).

IN VIVO STUDIES

The 2005 report also showed that therapeutic efficacy studies indicated a progressive decline in efficacy after 5 and 7 days of treatment with artemisinin-based monotherapies in China and Viet Nam. Many cases of 'resistance' were reported in the literature but not properly documented; these included failures after short treatment, early deaths from cerebral malaria, absence of PCR data to eliminate reinfection and insufficiently documented drug quality control (Jena et al., 1997; Das et al., 2000; Gogtay et al., 2000; Sahr et al., 2001; Singh, 2002). Most of the failures were late treatment failures, but two case reports who failed to respond to a 7-day oral artesunate treatment in Thailand were published (Luxemburger et al., 1998).

CLINICAL TRIAL IN TASANH, CAMBODIA

The first clear, well-documented evidence of artemisinin resistance was provided in a study of oral artesunate monotherapy conducted in Cambodia by the Armed Forces Research Institute of Medical Sciences (Noedl et al., 2008). Patients presenting to malaria clinics in Tasanh, Battambang Province (south of Pailin Province), were admitted to hospital for 28 days, where they were treated with either oral artesunate monotherapy (4 mg/kg body weight per day for 7 days) or quinine (30 mg/kg body weight per day) plus tetracycline (25 mg/kg body weight per day) in a split dose every 8 h for 7 days. Four treatment failures were observed among the 60 patients who received artesunate. Two of these four patients had adequate drug plasma levels and were consequently classified as having artesunate-resistant infections. They had parasite clearance times of 133 h and 95 h, which are markedly longer than the median parasite clearance time of 52.2 h. The IC_{50} for dihydroartemisinin for parasites isolated from these patients was four times the IC_{50} geometric mean for cured patients and almost 10 times that for the reference clone W2. During follow-up it was observed that 47.9% of the patients who had received oral artesunate monotherapy still had parasites at 48 h, and 21.9% still had parasites at 72 h. Although only two (3.3%) of the treatment failures met the criteria for artemisinin resistance defined by the authors, these results confirmed those of studies conducted between 2002 and 2004 by the National Centre for Parasitology, Entomology and Malaria Control in Cambodia.

BAN ON ORAL MONOTHERAPY

As a consequence, WHO called for increased, vigilant monitoring in the Greater Mekong subregion and also took action to minimize activities that could reduce the sensitivity of *P. falciparum* to artemisinin. In January 2006, WHO urged the pharmaceutical industry to stop the sale of single-dose forms of artemisinins and began monitoring manufacturers of oral artemisinin-based monotherapies and the availability of these drugs in countries. WHO called attention to the risks of resistance to artemisinins, citing the reduced sensitivity in the Greater Mekong subregion as a possible consequence of the use of oral monotherapy (Rehwagen, 2006). In addition, in May 2007, the World Health Assembly adopted a resolution urging Member States to "cease progressively the provision in both the public and private sectors of oral artemisinin monotherapies".

WHO CONSULTATION, PHNOM PENH, JANUARY 2007

As a response to the results of the studies conducted on the Cambodia–Thailand border, WHO organized a consultation in Phnom Penh in January 2007 (WHO, 2007), which was attended by representatives of the ministries of health of the countries concerned and international research institutes. Great concern was expressed about the delay in parasite clearance, as a distinctive property of artemisinins is their ability to reduce most of the parasite biomass in a short time; therefore, loss of this activity would have significant implications for the overall effectiveness of ACTs. Further, if a high proportion of parasites still remains on day 3, increased drug pressure is placed on the partner drug, making it also more vulnerable to resistance. The consultation concluded that, to determine whether there had indeed been a change in the efficacy of the artemisinin component, further clinical studies of oral artesunate monotherapy were needed. Despite some remaining questions, it was agreed that, given the threat that artemisinin resistance would pose to global malaria control, it would be negligent to wait for more evidence before planning for containment.

4.3 Pilot project to confirm artemisinin resistance

PROJECT DESCRIPTION

In November 2007, the Bill & Melinda Gates Foundation provided funding to the WHO Global Malaria Programme to coordinate a project entitled 'Artemisinin resistance: pilot studies to confirm, characterize, and plan for containment (ARC3)'. The activities included four clinical trials, in vitro, pharmacokinetics and molecular markers studies, and a drug quality study. The institutions involved in ARC3 are listed in Box 5.

BOX 5. ARC3 COLLABORATING INSTITUTIONS

- Armed Forces Research Institute of Medical Sciences, Bangkok, Thailand
- Bureau of Vector Borne Disease, Bangkok, Thailand
- Institut Pasteur du Cambodge, Phnom Penh, Cambodia
- Mahidol–Oxford Tropical Medicine Research Unit, Bangkok, Thailand
- Medical University of Vienna, Austria / Malaria Research Initiative Bandarban, Bangladesh
- National Centre for Parasitology, Entomology and Malaria Control, Phnom Penh, Cambodia
- Shoklo Malaria Research Unit, Mae Sot, Thailand
- United States Pharmacopeia, Rockville, Maryland, USA
- University of Maryland School of Medicine, Baltimore, Maryland, USA
- University of South Florida, Tampa, Florida, USA
- WHO Malaria Mekong Programme, Bangkok, Thailand
- WHO Regional Office for South-East Asia, New Dehli, India
- WHO Regional Office for the Western Pacific, Manila, Philippines

The objectives of the ARC3 project were to:

- confirm clinically relevant artemisinin resistance;
- if clinical resistance is confirmed, further characterize the resistance to define in vitro phenotypes and genotypes for use in global surveillance for artemisinin resistance;
- establish the prevalence of substandard and counterfeit drugs on the Cambodia–Thailand border; and
- develop strategies to combat the spread of artemisinin-resistant malaria in the Greater Mekong subregion and internationally.

The clinical trials were supported by the national malaria control programmes of each country. The efficacy of artesunate–mefloquine and oral artesunate monotherapy was studied in Bangladesh, Cambodia and Thailand; the sites and treatment arms are listed in Table 3. The blood samples from the clinical studies were analysed at the Mahidol–Oxford Tropical Medicine Research Unit, the Armed Forces Research Institute of Medical Sciences, the Institut Pasteur du Cambodge, the University of Maryland and the University of South Florida to identify changes in parasite sensitivity. Research was conducted to determine whether the genotype and phenotype abnormalities linked to drug resistance could be detected. Plasma drug concentrations were measured during treatment to analyse the pharmacokinetics of artesunate and

dihydroartemisinin. The availability of substandard drugs and counterfeit products along the Cambodia–Thailand border was assessed in a household survey and a drug quality study.

TABLE 3. ARC3 clinical studies (2007–2009)

INSTITUTION	STUDY SITE	TREATMENT ARMS	FOLLOW-UP (DAYS)
Armed Forces Research Institute of Medical Sciences	Tasanh, Battambang Province, Cambodia	(1) artesunate 2 mg/kg bw per day for 7 days (2) artesunate 4 mg/kg bw per day for 7 days (3) artesunate 6 mg/kg bw per day for 7 days	42
Mahidol–Oxford Tropical Medicine Research Unit	Pailin, Pailin Province, Cambodia	(1) artesunate 2 mg/kg bw per day for 7 days (2) artesunate 4 mg/kg bw per day for 3 days and a split dose of mefloquine: 15 mg/kg bw on day 3, 10 mg/kg bw on day 4	63
Shoklo Malaria Research Unit	Wang Pha, Tak Province, Thailand	(1) artesunate 2 mg/kg bw per day for 7 days (2) artesunate 4 mg/kg bw per day for 3 days and a split dose of mefloquine: 15 mg/kg bw on day 3, 10 mg/kg bw on day 4	63
Medical University of Vienna and Malaria Research Initiative Bandarban	Bandarban, Bangladesh	(1) artesunate 2 mg/kg bw per day for 7 days (2) artesunate 4 mg/kg bw per day for 7 days (3) quinine–doxycycline for 7 days	42

bw, body weight.

RESULTS

The median parasite clearance time was markedly slower in Pailin (84 h) than in Wang Pha (48 h) ($p < 0.001$) (Dondorp et al., 2009). Neither increasing nor splitting the dose of artesunate improved the parasite clearance time in Palin, although further research is still needed to confirm these findings. Severe neutropenia was observed 1 week after the end of the treatment in patients who received a dose of 6 mg/kg body weight of artesunate monotherapy over 7 days (Bethell et al., 2010). A prolonged parasite clearance time appears to be a heritable trait in Cambodia (Anderson et al., 2010).

A poor correlation between the in vivo phenotype and the in vitro standard was found in the clinical trials, perhaps because the methods used to measure in vitro values were not designed to detect artemisinin resistance. New in vitro tools are therefore needed. In addition, there was no correlation between a number of different mutations (*PfATPase6* or *Pfmdr1* or mitochondrial genome) or *Pfmdr1* copy number and in vivo phenotypes in samples from Wang Pha and Pailin (Imwong et al., 2010).

Research on the mechanisms of artemisinin resistance has focused on changes in parasite clearance in vitro. Persistence of temporarily growth-arrested young trophozoites, which awaken from dormancy days or weeks later, has been reported (Teuscher et al., 2010). In addition, a high dose of artemisinin was shown to induce developmental arrest in a subpopulation of ring stages (Witkowski et al., 2010c).

Genome-wide studies of associations and studies of genomic signatures of selection are under way with next-generation genome sequencing and a single nucleotide polymorphism genotyping platform in order to identify genetic polymorphisms associated with prolonged parasite clearance. If candidate resistance markers are identified, they will be validated in the field for use in surveillance of resistance.

The information collected in the ARC3 studies led the investigators to conclude that the proportion of patients who are still parasitaemic on day 3 (72 h after the beginning of the treatment) in clinical trials with ACTs is the most appropriate indicator of possible artemisinin resistance.

4.4 Defining artemisinin resistance

TOOLS AND MEASURES OF ARTEMISININ RESISTANCE

Several definitions of artemisinin resistance have been proposed (Box 6). Different definitions may be useful for different purposes, such as making policy decisions or addressing specific research questions.

BOX 6. DEFINITION OF ARTEMISININ RESISTANCE

Only patients who meet the following criteria are classified as having an artemisinin-resistant infection:

- persistence of parasites 7 days after treatment or recrudescence within 28 days after the start of treatment (artemisinin-based monotherapy),
- adequate plasma concentration of dihydroartemisinin,
- prolonged time to parasite clearance and
- reduced in vitro susceptibility to dihydroartemisinin (Noedl, 2005).

Markedly prolonged parasite clearance time (Dondorp et al., 2009).

In the remainder of the document, the term 'artemisinin resistance' is a working definition used to refer to:

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

* This definition may be prone to confounding factors (known and unknown) such as splenectomy, haemoglobin abnormalities and reduced immunity.

The unique ability of artemisinins to clear parasites rapidly is well known and has been considered their 'pharmacodynamic hallmark' (White, 2008). While their mechanism of action is not fully understood, they are active against both asexual and sexual stages of parasite development, with the greatest activity against the blood stages, including the young ring stages. The proportion of patients who are parasitaemic on day 3 after treatment with an ACT has been found to be a suitable tool for screening for artemisinin resistance (Stepniewska et al., 2010). Failure to clear parasites by day 3 indicates a change in the pattern of parasite susceptibility to artemisinins and is probably the first stage of artemisinin resistance, which is thought to be associated with loss of activity against the early ring stages (Dondorp et al., 2009).

In a meta-analysis of 85 randomized controlled trials conducted in 25 countries, data on parasite clearance by over 18 000 patients with falciparum malaria treated with artemisinin derivatives were examined (Stepniewska et al., 2010). This study demonstrated the influence of baseline parasite density on parasite clearance time. Artemisinin resistance was considered highly unlikely if < 3% of patients with a baseline parasite density < 100 000 per µl who received the recommended 3-day treatment with an ACT were parasitaemic on day 3. The following caveats should nevertheless be considered:

- The threshold of 3% should be considered a 'rule-out' threshold, below which artemisinin resistance is highly unlikely.
- This meta-analysis and clinical studies conducted in the Greater Mekong subregion show that the prevalence of parasitaemia on day 3 differs for artemisinins and for ACTs because of the activity of the partner drug. Consequently, comparisons between these studies should be made with caution. The results may also differ according to the artemisinins and the partner drugs used.

- In routine monitoring, day 3 may not necessarily correspond to exactly 72 h of treatment. The indicator should therefore be standardized to avoid overestimating the proportion of patients parasitaemic on day 3.
- Assessment of low density parasitaemia at 72 h depends on the sensitivity of microscopy, as such slides are easy to misread.

The ARC3 investigators proposed that early signs of artemisinin resistance first be detected in therapeutic efficacy studies of ACTs conducted according to a standard protocol. A threshold of 10% of parasitaemic patients on day 3 was established as appropriate for initiating studies of oral artesunate monotherapy. The information that should be collected or calculated in studies of oral artesunate monotherapy are the proportion of patients with treatment failure by day 28 or 42, the proportion of patients who are parasitaemic on day 3, pharmacokinetics, parasite clearance time, parasite reduction ratio at 48 h and the slope of the log–linear parasite clearance curve. In addition, the investigators agreed that a definitive threshold for artemisinin resistance (based on the proportion of patients parasitaemic on day 3) can be established only in more extensive studies with both artemisinins and ACTs. Sites at which ≥ 3% but < 10% of patients are parasitaemic are considered as being in a 'grey' zone, in which caution and increased scrutiny are required.

In low-transmission settings, the same parasite strain is often found in many patients enrolled in the same study. Genetically identical parasites can be identified by microsatellite genetic marker studies. The finding of clusters of the same genotyped strain in patients with slow parasite clearance rates is perhaps the most precise indication of artemisinin-resistant parasite strains.

Confirmation of artemisinin resistance during routine surveillance is difficult, as most studies are conducted to determine the efficacy of ACTs and not of one of the artemisinins alone. Absolute confirmation of artemisinin resistance can be established only when artemisinins are used alone, preferably with measures of the artemisinin blood concentration. A genetic marker of artemisinin resistance has not yet been identified, so that it is impossible to confirm the presence of artemisinin resistance in a molecular marker study.

CONSEQUENCES OF ARTEMISININ RESISTANCE

Reduced sensitivity of falciparum malaria to artemisinins will pose new challenges for patient follow-up. Clinical resolution might be slightly prolonged, potentially leading to dissatisfied patients and inappropriate treatment practices. Prolongation of parasite clearance might also affect the outcome of severe and complicated malaria treated with injectable artemisinins. In the ARC3 studies of oral artesunate monotherapy, some patients who presented with both fever and parasitaemia on day 3 were found during follow-up to have resolved both their symptoms and parasitaemia spontaneously, without rescue treatment (Dondorp et al., 2009; D. Bethell, unpublished data). Clinicians should therefore be aware that 'artificial' early treatment failures may occur in studies of oral artesunate monotherapy in areas of artemisinin resistance, which can result in an overestimate of the true failure rate.

Increased parasite clearance time with oral artemisinin-based monotherapies and ACTs has been associated with increased gametocyte carriage (Carrara et al., 2009). An increased incidence of infections with patent gametocytaemia may in turn increase the risk for transmission of less sensitive parasites; however, the implications for transmission are not yet well understood. Data on the transmissibility of such parasites, including the sporogonic cycle and the infectivity of the resulting sporozoites, are not yet available.

The decreasing efficacy of artemisinins is alarming because it suggests that they may no longer be able to fulfill their key role in combination therapy of reducing the parasite biomass and protecting the partner drug. A longer parasite clearance time will result in a greater parasite biomass for a longer time, which may increase the odds of de novo resistance mutations to the partner drug. Once such de novo mutations emerge, they will favour the parasites' survival under (partner) drug pressure. Thus, the declining efficacy of artemisinins could also jeopardize the partner medicines. It is therefore essential to develop alternatives to the artemisinin component of the combinations and to continue to develop new partner drugs.

For patients with severe malaria, WHO recommends injectable artemisinin-based monotherapies, in particular artesunate, as one of the options for initial treatment (WHO, 2010). In areas where artemisinin resistance is confirmed, however, administration of parenteral artemisinin-based monotherapy may no longer be appropriate. In order to determine the efficacy of this treatment in the context of artemisinin resistance, clinical trials in areas where artemisinin resistance has been confirmed should monitor both the efficacy of injectable artemisinin-based monotherapies and the case fatality rate among patients with severe malaria. In routine practice, in which oral treatment is administered as soon as the patient is able to tolerate oral treatment, neither the proportion of patients with parasitaemia on day 3 nor the efficacy of a 7-day artemisinin treatment can be systematically assessed. In these cases, the parasite reduction rate over the first 48 h should be recorded, and all severe malaria cases should be reported to a central database, so that changes in treatment response can be detected over time.

4.5 Current situation

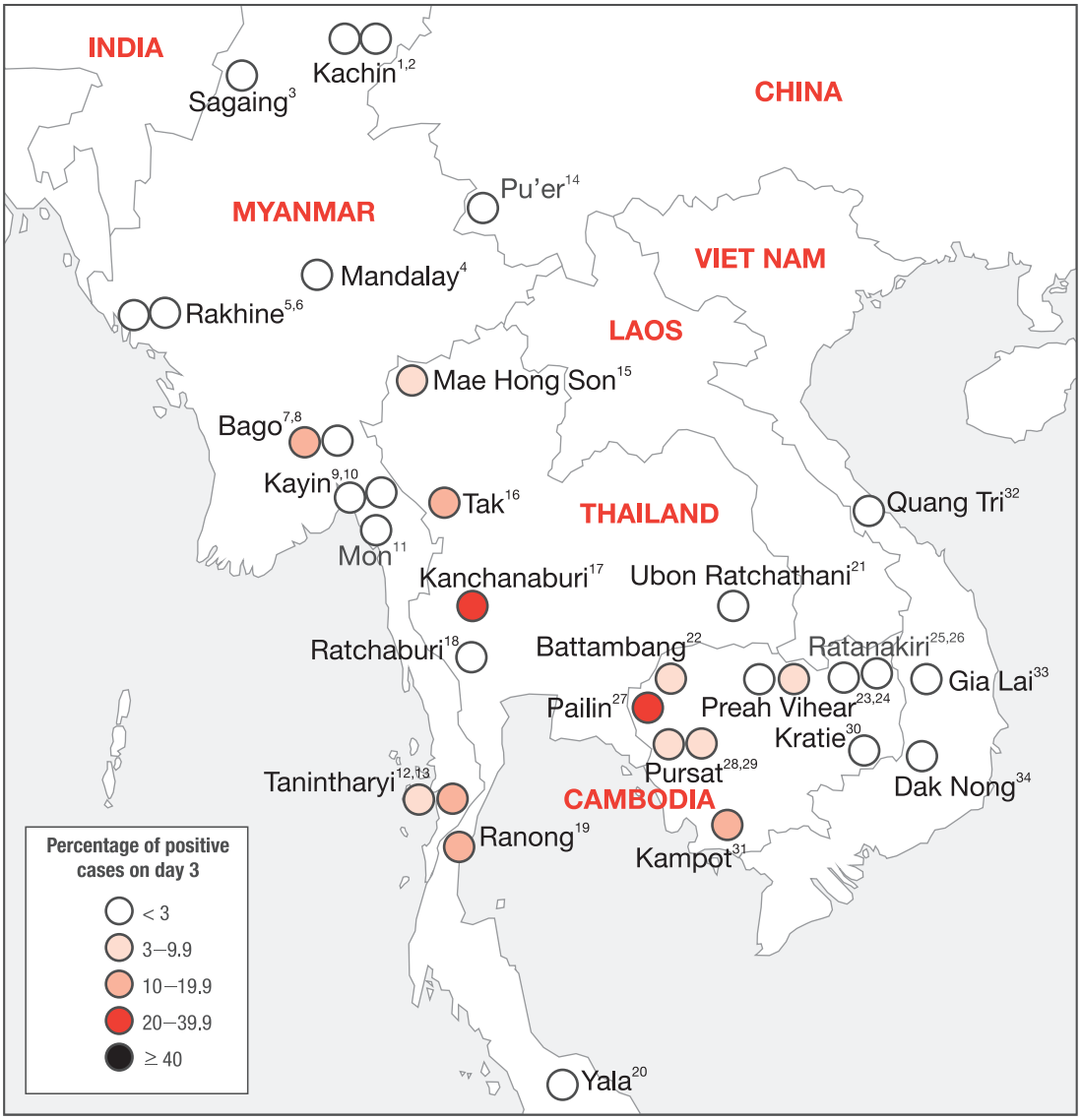
The severity of artemisinin resistance varies across the Greater Mekong subregion. The current working definition of artemisinin resistance is based on parasite clearance, as observed during therapeutic efficacy studies of ACTs and oral artesunate monotherapy (Figures 24 and 25).

The strongest evidence of artesunate resistance is found in northwest Cambodia, at the Thai border, where the proportions of patients still parasitaemic after 3 days of ACT or oral artesunate monotherapy are the highest ever reported. In Pailin, the proportion of patients who were still parasitaemic after 3 days of treatment with dihydroartemisinin–piperaquine increased from 26% to 33% between 2008 and 2009. In Pailin and Tasanh, over 40% of patients were parasitaemic on day 3 after treatment with 2–4 mg/kg body weight per day of oral artesunate monotherapy. A few late treatment failures were observed at both sites in the presence of an adequate plasma concentration of artesunate or dihydroartemisinin and, in some cases, with correspondingly reduced in vitro susceptibility to dihydroartemisinin. Further, the heritable trait observed at the Cambodia–Thailand border indicates that the slow clearance rate is due to changes in parasite genetics, suggesting that this phenomenon could spread from the focus of its origin to contiguous parasite populations if it is not contained (Anderson et al., 2010).

Elsewhere in the region, the situation is less severe but still merits careful monitoring and early response. At the Myanmar–Thailand border, 10–20% of patients have been found to be parasitaemic after a 3-day treatment with ACTs. At the China–Myanmar border, studies of oral artesunate monotherapy (16 mg/kg body weight per day over 7 days) showed that 25% of patients were still parasitaemic at day 3.

The failure rate with artesunate monotherapy remains low, even if the proportion of patients who are parasitaemic at day 3 is very high. Surprisingly, the failure rate of 7-day oral artesunate monotherapy in Cambodia (Tasanh), Thailand (Wang Pha) and at the China–Myanmar border was < 10% after a follow-up of 28–63 days, although the percentage of patients who were parasitaemic on day 3 was > 50% in Pailin and 25% at the China–Myanmar border. At this early stage, it is not known whether artemisinin resistance will be limited to an increasing prevalence of delayed parasite clearance and gametocyte carriage, or if high rates of late treatment failure will also eventually be seen. There are, however, two exceptions.

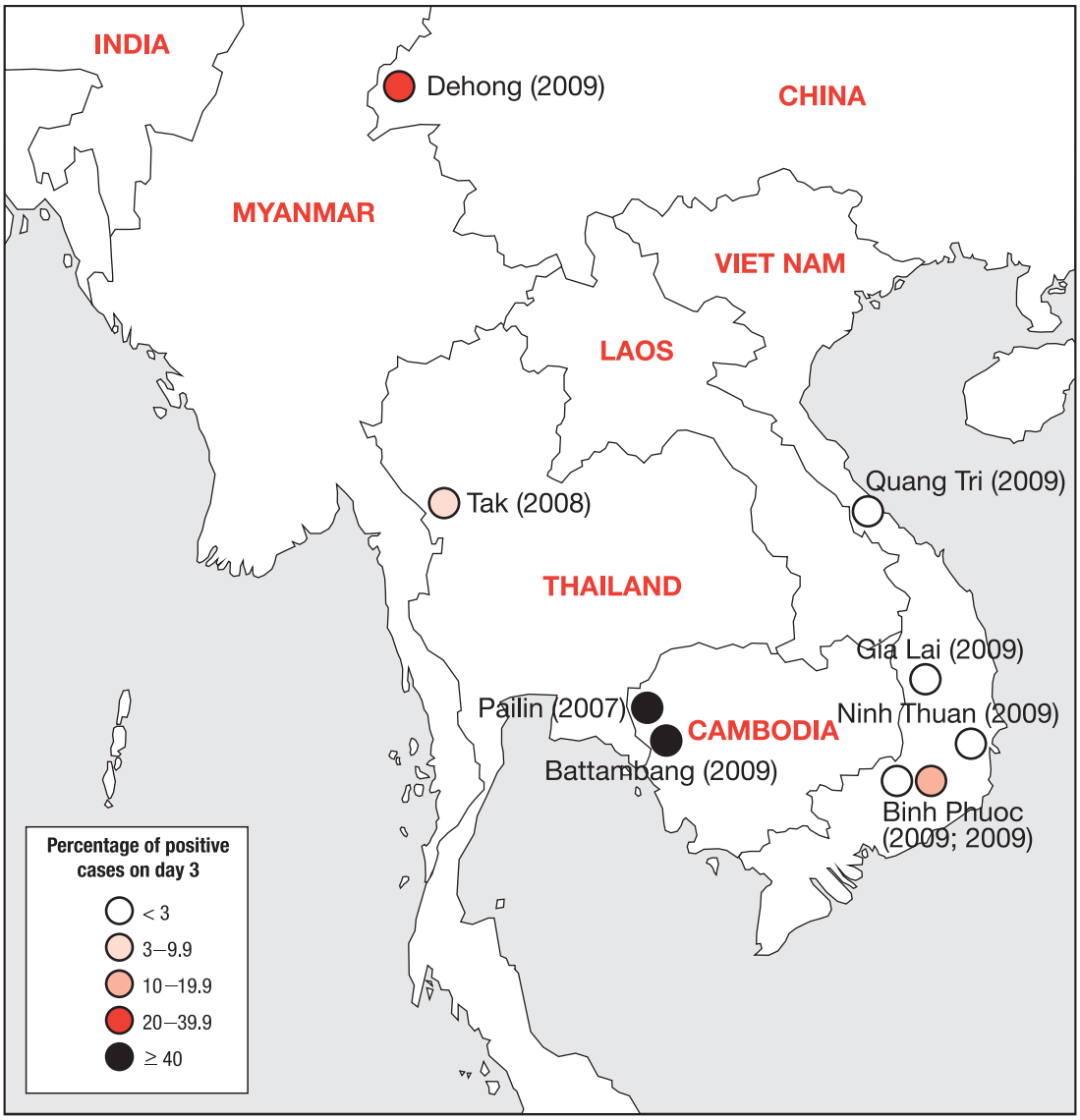
FIGURE 24. Percentages of patients with *P. falciparum* parasitaemia on day 3 after treatment with an ACT (2006–2010)



The map shows the results of the most recent therapeutic efficacy study per site and per drug only: 1. 2007, artemether–lumefantrine; 2. 2007, artesunate–amodiaquine; 3. 2009, artemether–lumefantrine; 4. 2007, artesunate–amodiaquine; 5. 2007, artemether–lumefantrine; 6. 2007, artesunate–amodiaquine; 7. 2009, artemether–lumefantrine; 8. 2009, dihydroartemisinin–piperaquine; 9. 2007, artemether–lumefantrine; 10. 2007, artesunate–amodiaquine; 11. 2007, artemether–lumefantrine; 12. 2009, artemether–lumefantrine; 13. 2009, dihydroartemisinin–piperaquine; 14. 2009, dihydroartemisinin–piperaquine; 15. 2009, artesunate–mefloquine; 16. 2009, artesunate–mefloquine; 17. 2009, artesunate–mefloquine; 18. 2008, artesunate–mefloquine; 19. 2009, artesunate–mefloquine; 20. 2008, artesunate–mefloquine; 21. 2008, artesunate–mefloquine; 22. 2007, artesunate–mefloquine; 23. 2010, artesunate–mefloquine; 24. 2009, dihydroartemisinin–piperaquine; 25. 2006, artesunate–mefloquine; 26; 2010, dihydroartemisinin–piperaquine; 27. 2010, dihydroartemisinin–piperaquine; 28. 2007, artesunate–mefloquine; 29. 2009, dihydroartemisinin–piperaquine; 30. 2006, artesunate–mefloquine; 31. 2008, artesunate–mefloquine; 32. 2008, dihydroartemisinin–piperaquine; 33. 2009, dihydroartemisinin–piperaquine; 34. 2010, dihydroartemisinin–piperaquine.

In Pailin, the treatment failure rate following treatment with 7-day artesunate was as high as 30% (6/20 patients); however, the sample size was small and the eligibility criteria for this study allowed the inclusion of patients with a parasitaemia up to 200 000 per µl, while the recommendation for routine monitoring in areas of low transmission is < 100 000 per µl (WHO, 2009a). Moreover, some patients presenting with a late treatment failure were found to have an insufficient blood concentration of artesunate or dihydroartemisinin.

FIGURE 25. Percentages of patients with *P. falciparum* parasitaemia on day 3 after treatment with oral artesunate monotherapy (2–4 mg/kg body weight per day), 2007–2009



The map shows the results of the most recent therapeutic efficacy study per site and per drug only.

Reduced artemisinin susceptibility was also reported in Bu Dang district, Binh Phouc Province, in Viet Nam. The percentage of patients who were parasitaemic on day 3 after treatment with an ACT or 7-day oral artesunate monotherapy treatment was reported to be > 10%. In addition, a relatively high rate of treatment failure was found after a total dose of 16 mg/kg body weight per day of artesunate over 7 days. The focus seems to be limited to one district, as studies in the neighbouring district, Phuoc Long, in the same province, showed a low percentage (< 3%) of patients who were parasitaemic on day 3 and no treatment failure after 7 days of artemisinin or artesunate monotherapy and 28 days of follow-up (Thanh et al., 2010). Binh Phuoc is the only province in Viet Nam where reduced susceptibility to artemisinins has been reported. Additional studies, including pharmacokinetics, molecular markers and in vitro studies, are under way.

These results indicate a clear change in the sensitivity of parasites to artemisinins. Diligent monitoring will be required in this region, as these subtle changes can be detected only on careful analysis of data on therapeutic efficacy. Despite the observed changes in parasite sensitivity to artemisinins, the treatment failure rates with ACTs remain low (< 10%), provided that partner drugs that are effective in the region are selected and used (Figure 26). High treatment failure rates with ACTs have been observed only in those areas where resistance to a partner drug has been confirmed. In those settings, changing to an ACT with a different partner drug resulted in high treatment efficacy. Therefore, the term 'ACT resistance' should be avoided. When the efficacy of an ACT appears to be compromised, reference should be made to that ACT and not to ACTs in general.

4.6 Containment of artemisinin-resistant malaria parasites

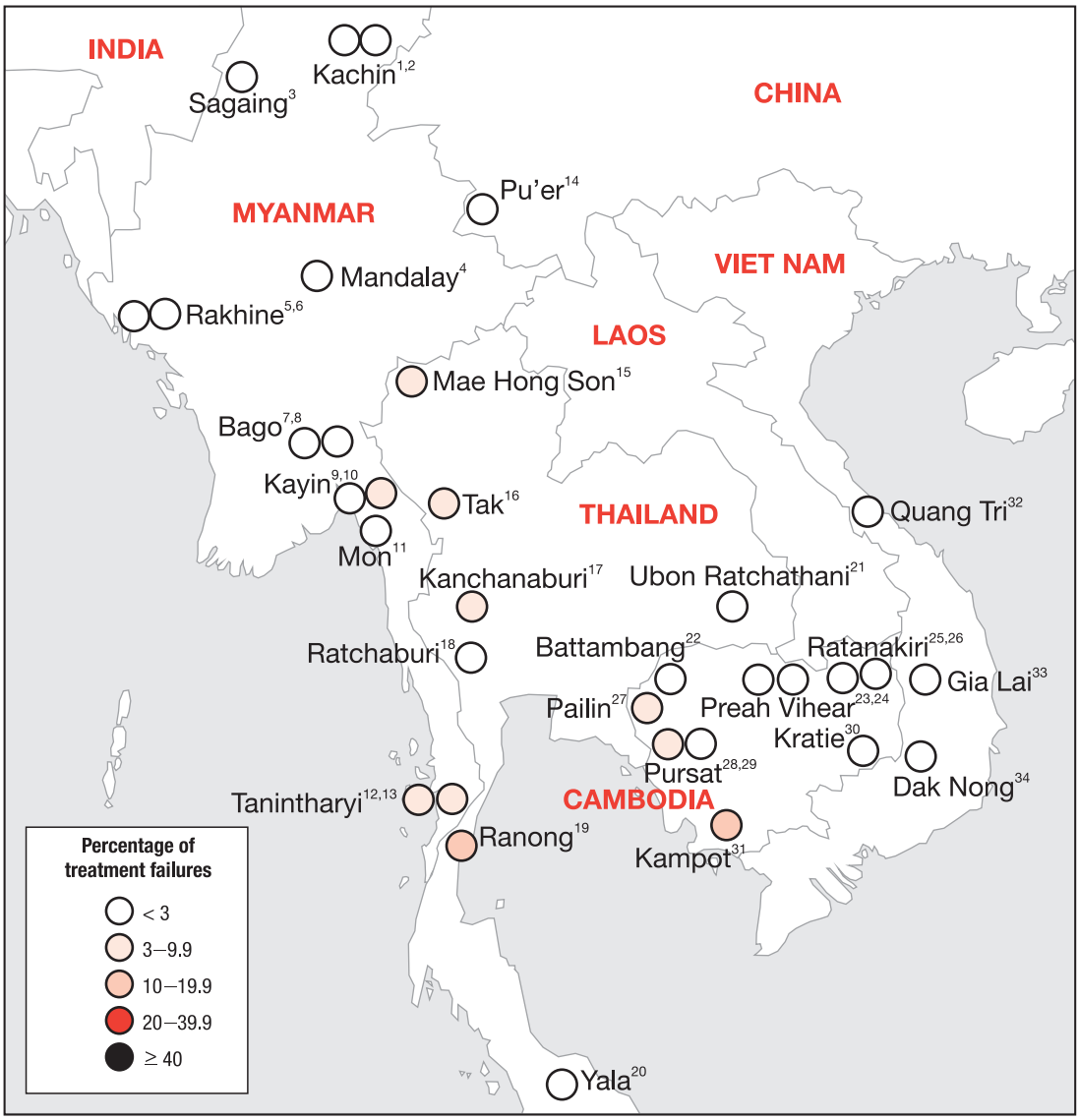
A containment strategy was devised by experts in antimalarial drug resistance who met in early 2008 to define the optimal technical strategies for containing the transmission of drug-resistant malaria, outline an operational containment plan and set priorities for research on the basis of gaps in knowledge. They agreed that the strategy should include improved case management, with early diagnosis and treatment, and intensive vector control for reducing transmission. Activities should be targeted to both fixed and mobile populations. The project should also include measures to combat use of counterfeit and substandard drugs and a communication strategy to encourage appropriate antimalarial drug use. An operational research component was included, to clarify the extent of the spread and to identify the reasons for the emergence of resistance. A detailed account of the discussions leading to consensus on the containment strategy can be found in the meeting reports (WHO, 2008b; WHO, 2008c; WHO, 2008d).

The project was designed to take into account several operational challenges: defining the population at risk in an area of high population mobility, providing access to diagnosis and treatment within weak public health infrastructures and engaging with the private sector (licensed drug outlets and providers). Rapid scaling-up of containment would require the close involvement of nongovernmental organizations and communities. Furthermore, containment efforts should be harmonized across borders and should address urgent issues including establishing an organizational structure to facilitate a rapid, coordinated, effective response; intensifying vector control in the target area; ensuring deployment in both the public and private sectors; removing artemisinin monotherapies; and replacing co-blistered ACTs with an effective co-formulated antimalarial drug.

In November 2008, the Bill & Melinda Gates Foundation committed US$ 22.5 million towards the containment project. Additional contributions were made by the Global Fund to Fight AIDS, Tuberculosis and Malaria and the United States Agency for International Development. The goal of the project was to stop the spread of artemisinin-resistant parasites by removing selection pressure and by ultimately eliminating *P. falciparum*-resistant parasites. The containment project was ambitious: like ARC3, it would require the effective collaboration of dedicated experts and partners. The objectives are listed in Box 7, and a detailed summary of the activities is available at http://www.who.int/malaria/diagnosis_treatment/resistance/en/index.html.

The containment project is currently ongoing in certain zones on the Cambodia–Thailand border area. In zone 1, where artemisinin resistance has already been detected, intensive activities are aimed at local elimination of *P. falciparum*. In Cambodia, zone 1 covers about 270 000 people in four provinces (all of Pailin and parts of Battambang, Pursat and Kampot). In Thailand, about 110 000 people live in zone 1 in the border areas of Trat and Chantaburi provinces. Zone 2 borders zone 1, and its residents are considered at high risk for infection by artemisinin-resistant parasites. In Cambodia, zone 2 covers nine provinces with a total population of more than 4 million (excluding towns). In Thailand, zone 2 comprises seven provinces with a population of nearly 7 million, about 150 000 of whom live in areas at risk for malaria.

FIGURE 26. Failure rates on day 28 after treatment with an ACT, 2006–2010



The map shows the results of the most recent therapeutic efficacy study per site and per drug only: 1. 2007, artemether–lumefantrine; 2. 2007, artesunate–amodiaquine; 3. 2009, artemether–lumefantrine; 4. 2007, artesunate–amodiaquine; 5. 2007, artemether–lumefantrine; 6. 2007, artesunate–amodiaquine; 7. 2009, artemether–lumefantrine; 8. 2009, dihydroartemisinin–piperaquine; 9. 2007, artemether–lumefantrine; 10. 2007, artesunate–amodiaquine; 11. 2007, artemether–lumefantrine; 12. 2009, artemether–lumefantrine; 13. 2009, dihydroartemisinin–piperaquine; 14. 2009, dihydroartemisinin–piperaquine; 15. 2009, artesunate–mefloquine; 16. 2009, artesunate–mefloquine; 17. 2009, artesunate–mefloquine; 18. 2008, artesunate–mefloquine; 19. 2009, artesunate–mefloquine; 20. 2008, artesunate–mefloquine; 21. 2008, artesunate–mefloquine; 22. 2007, artesunate–mefloquine; 23. 2010, artesunate–mefloquine; 24. 2009, dihydroartemisinin–piperaquine; 25. 2006, artesunate–mefloquine; 26; 2010, dihydroartemisinin–piperaquine; 27. 2010, dihydroartemisinin–piperaquine; 28. 2007, artesunate–mefloquine; 29. 2009, dihydroartemisinin–piperaquine; 30. 2006, artesunate–mefloquine; 31. 2008, artesunate–mefloquine; 32. 2008, dihydroartemisinin–piperaquine; 33. 2009, dihydroartemisinin–piperaquine; 34. 2010, dihydroartemisinin–piperaquine.

The primary containment activities are aggressive vector control and effective case management. More than 500 000 long-lasting insecticide-treated mosquito nets have been distributed, and more than 200 000 existing nets have been re-treated. The distribution campaign resulted in 100% coverage in zone 1 and in high-risk villages in zone 2. Many village health care workers were recruited and trained in order to improve case detection and treatment; they are equipped to provide free screening with a rapid diagnostic test, and patients with a positive test result for malaria receive free treatment and follow-up. In Thailand, all malaria cases are followed up for 28 days. In Cambodia, the patients are monitored during the 3 days of treatment, and patients who are still parasitaemic after day 3 are followed for up to 28 days. The provision of free treatment and care helps to undermine the sale of counterfeit and substandard antimalarial drugs by the private sector. Treatment is available at health facilities established to diagnose and treat malaria, which are open 24 h a day.

BOX 7. OBJECTIVES OF THE ARTEMISININ RESISTANCE CONTAINMENT PROJECT

- to eliminate artemisinin-resistant parasites by detecting all malaria cases in target areas and ensuring effective treatment and gametocyte clearance;
- to decrease drug pressure for selection of artemisinin-resistant malaria parasites (including the ban on monotherapy);
- to prevent transmission of artemisinin-resistant malaria parasites by mosquito control and personal protection;
- to limit the spread of artemisinin-resistant malaria parasites by mobile and migrant populations;
- to support containment and elimination of artemisinin-resistant parasites by comprehensive behaviour change communication, community mobilization and advocacy;
- to undertake basic and operational research to fill knowledge gaps and ensure that the strategies applied are evidence-based; and
- to provide effective management, surveillance and coordination for rapid, high-quality implementation of the strategy.

In addition to vector control and case management, education programmes inform villagers about the importance of using insecticide-treated mosquito nets for the prevention of malaria and about appropriate treatment to prevent the spread of drug resistance. The campaign includes posters, brochures, street theatre, village meetings, radio and television advertisements. This programme will continue throughout the project. A special campaign has been launched to include the mobile population in containment. Migrants come to the Cambodia–Thailand border area to work on farms and construction projects and in military postings, forestry, land development and gem mining. The strategies used to include the mobile population in containment activities include:

- engaging mobile malaria workers to seek out transient workers;
- providing mosquito nets to farm owners for distribution to seasonal workers;
- establishing diagnosis and treatment stations in construction camps and temporary villages of military families;
- operating mobile malaria clinics at all border crossings; and
- providing all education materials in both Thai and Khmer and designing them to ensure that the messages and appearance are the same in both languages.

Efforts have also been made to stop the sale of counterfeit and substandard drugs, which are a major factor in the development of resistance. The Government of Cambodia has prohibited the sale of oral artemisinin-based monotherapies, and the ban is enforced by justice police, who systematically visit pharmacies, shops and markets. Workshops and education materials are used to inform both medicine sellers and residents.

Research on the emergence and spread of artemisinin resistance includes a pilot project involving intense screening and treatment in 20 villages in Cambodia that are most severely affected by malaria. The goal is to identify and treat all people infected with *P. falciparum*, including those who are asymptomatic. Screening and follow-up, with epidemiological investigation, provides important information about resistant parasites and the risk of their spread. The results of these early interventions will be the basis for future activities.

Routine monitoring and clinical trials to confirm artemisinin resistance are under way in the Greater Mekong subregion, and studies of the efficacy of ACTs have been intensified in other parts of the world. In view of the situation in the Greater Mekong, action is needed, as there are no alternative antimalarial treatments that could replace artemisinins. The difficulty being experienced in defining artemisinin resistance should not delay the planning and implementation of containment activities in areas where artemisinin resistance is suspected. For example, extending the containment strategy to Viet Nam and to the Myanmar–Thailand border is being discussed.

The evidence of artemisinin resistance in the Greater Mekong subregion indicates that it is time to define a global strategy to prevent the spread of artemisinin resistance. History has shown that once resistance to antimalarial treatment emerges it is only a question of time before it spreads. For the first time, the global malaria community has the opportunity to contain resistance before it spreads. The WHO Global Malaria Programme has engaged a large, diverse group of stakeholders from the global malaria community to develop a global response, which will be published in a document entitled *Global plan for artemisinin resistance containment*.

5. Challenges to monitoring antimalarial drug efficacy

Routine monitoring of the efficacy of antimalarial drugs is necessary for effective case management and early detection of resistance. When studies are conducted according to a standard protocol, the results can be used as a basis for national treatment policy and for global surveillance of antimalarial drug efficacy. There are, however, several challenges to conducting efficacy studies regularly.

According to the WHO standard protocol for monitoring antimalarial drug efficacy (WHO, 2009a), the efficacy of first- and second-line medicines should be tested at least once every 24 months at all sentinel sites. The sentinel sites should represent all epidemiological strata in a country. In addition, monitoring should be conducted at the same sentinel sites over time in order to allow analysis of trends. Currently, few countries are conducting an adequate number of studies. Between 2008 and 2009, therapeutic efficacy studies were performed in only 31 of 92 (33.7%) countries endemic for *P. falciparum* malaria.

The sense of urgency for monitoring drug resistance has unfortunately diminished over the past 10 years. When ACTs were introduced, it was commonly believed that artemisinins were not vulnerable to resistance. The low frequency of studies is also due to operational constraints. As transmission rates and malaria incidence decline in low-transmission areas, it has become more difficult to find patients who meet the inclusion criteria; other countries have been unable to conduct efficacy studies because of political instability or a lack of dedicated funds. The new recommendations to increase the length of follow-up for some combinations to 42 days and to use PCR to distinguish between a recrudescence and a reinfection have increased the cost of therapeutic efficacy studies (WHO, 2008a; WHO, 2009a).

Studies should adhere to high quality standards, in accordance with the WHO protocol (WHO, 2009a). At a minimum, each study should include ethical clearance by an institutional review board or independent ethics committee; quality control of the drugs used in the study; quality control of the parasitological diagnoses by at least two trained microscopists per site or by an external review of slides; and accurate, complete case report forms entered onto a spreadsheet or into a database. Ideally, a clinical trial monitor will visit the study site(s) at least three times: once before the study for training, once during the study for monitoring and once at the end of the study for data validation. The monitor can help to prepare the study, supervise its conduct according to the protocols, ensure correct collection of data and help to analyse the data and write the report.

An effective global surveillance system for drug efficacy requires support and coordination at national and subregional levels. Taking the experience of the East African Network for Monitoring Antimalarial Treatment as its guide, WHO supported the creation of both national and subregional networks for monitoring antimalarial resistance (East African Network for Monitoring Antimalarial Treatment, 2001). The value of such networks is multiple. Information on therapeutic efficacy collected in these networks and experience with new drug combinations can be shared between countries in order to provide the best possible advice to ministries of health in selecting a new policy. Networks also allow more effective management of problems in border areas, where population movement may be intense; countries within the same network can decide to set up sentinel sites on both sides of a common border. The activities of such networks can include staff training (protocol implementation, microscopy, data analysis and validation, reporting and publication), training in the use of new tools, promoting cooperation between countries, encouraging countries to maintain monitoring, identifying regional trends and coordinating a regional response. All these activities can improve data quality.

Most of the regional networks were established in 2000–2001 to monitor the threat of antimalarial drug resistance. At one time, seven regional networks were monitoring drug efficacy with the technical or financial support of WHO (Box 8); however, in the absence of sustainable funding, four of the networks ceased functioning. Given the complexity of routine monitoring, reactivation of these inactive networks would help in the coordination, strengthening and support of global and national surveillance activities.

BOX 8. NETWORKS FOR MONITORING ANTIMALARIAL DRUG EFFICACY

Africa	• East African Network for Monitoring Antimalarial Treatment: Burundi, Kenya, Rwanda, Uganda, United Republic of Tanzania • Horn of Africa Network for Monitoring Antimalarial Treatment*: Djibouti, Eritrea, Ethiopia, Saudi Arabia, Somalia, Sudan, Yemen • Réseau d'Afrique Centrale pour le Traitement Antipaludique: Angola, Cameroon, Central African Republic, Chad, Congo, Democratic Republic of the Congo, Equatorial Guinea, Gabon, Sao Tome and Principe • West African Network for Monitoring Antimalarial Treatment 1: Cape Verde, Gambia, Guinea, Guinea-Bissau, Mauritania, Senegal • West African Network for Monitoring Antimalarial Treatment 2: Benin, Burkina Faso, Côte d'Ivoire, Ghana, Mali, Niger, Nigeria, Sierra Leone, Togo
Greater Mekong	• Mekong*: Cambodia, China, Lao People's Democratic Republic, Myanmar, Thailand, Viet Nam
South America	• Red Amazónica para la Vigilancia de la Resistencia a las Drogas Antimaláricas*: Bolivia (Plurinational State of), Brazil, Colombia, Ecuador, Guyana, Peru, Suriname, Venezuela (Bolivarian Republic of)

* Remains active.

The challenges associated with monitoring drug efficacy are significant, and, if they are not addressed, they will continue to impede the frequency and quality of drug efficacy monitoring. If regular monitoring of therapeutic drug efficacy is to be considered a priority in malaria-endemic countries, there must be a high level of support and commitment. Ultimately, political commitment and sustained investment are needed to enable national malaria control programmes and local research institutions to conduct therapeutic efficacy studies that are of consistently high quality. Capacity to conduct such studies and analyse and disseminate the data must be built into national malaria control programmes, in line with the recent renewed high-level global political commitment for investment in strengthening health systems in endemic countries.

Another challenge in drug efficacy monitoring is timely dissemination of study results. Data must be communicated rapidly in the context of the emergence of artemisinin resistance. Given the sensitivity of the data, efforts should be coordinated and the data managed by an independent, neutral organization established primarily to serve the interests of the countries.

A project based at the University of Oxford, United Kingdom of Great Britain and Northern Ireland, the WorldWide Antimalarial Resistance Network, has been launched to create a comprehensive, global, inclusive network to provide quality-assured information on antimalarial drug resistance (Sibley, Guerin & Ringwald, 2010). This network and the WHO Global Malaria Programme are collaborating since June 2009 in a 3-year pilot project involving the transfer of data and exchange of information on the development of tools to facilitate monitoring antimalarial drug efficacy and drug resistance.

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Annex 1. WHO global database on antimalarial drug efficacy

The global database on antimalarial drug efficacy was set up by WHO in 2000 to respond to the challenge posed by resistance of *P. falciparum* to antimalarial drugs. An up-to-date database on antimalarial drug efficacy is an invaluable source of information for people working on malaria, and specifically on drug resistance. It contains all the current, pertinent literature in one accessible location and allows analysis and filtering of the information as required.

The data in the database come from three main sources:

- published data, obtained by searching journal articles from MEDLINE, PubMed, Latin American and Caribbean Health Sciences Literature (LILACS), Scientific Electronic Library Online (SCIELO) and the Cochrane Library, and by systematic analysis of the reference lists in the selected articles;
- unpublished data from reports by ministries of health, national malaria control programmes, nongovernmental organizations, research institutes and partners involved in the development of new antimalarial medicines; and
- raw data from regular surveillance studies conducted according to the WHO standard protocol, incorporated into standardized files and sent to WHO for validation.

A1.1 Description

The WHO database contains the following information for each study of the therapeutic efficacy of an antimalarial drug against *P. falciparum*: year(s) and month(s) during which the study was conducted, name of the country and study site, medicines and dose used, the type of presentation (co-package or co-formulated), use of primaquine, the WHO protocol used and any modifications introduced to the standard protocol, duration of follow-up, quality control checks, additional studies (in vitro, molecular markers for drug resistance or measurements of drug concentration), percentage of patients lost to follow-up (including patients who were excluded or withdrew after enrolment), the number of patients eligible for the analysis, the percentages of adequate clinical and parasitological responses, early and late treatment failures calculated by the per-protocol and/or Kaplan-Meier method, the type of reference (publication, report, thesis, presentation, raw data), and a link to the webpage on which the reference was found.

All studies conducted between 1996 and June 2010 with a follow-up period of at least 14 days were entered into the database. Studies conducted according to the 1973, 1996, 2001 or 2009 WHO protocol were included in the database, despite differences in duration of follow-up and outcome classification. Studies conducted according to the 1973 WHO protocol were included because several studies have shown that the sum of early treatment failures, late clinical failures and late parasitological failures is equivalent to the sum of RI-, RII- and RIII-type failures (see Box 9), particularly in areas of low-to-moderate transmission. Studies that did not follow a WHO protocol but for which the failure and success rates were available were also entered in the database. Not all studies included in the database were used in the current analysis.

BOX 9. CLASSIFICATION OF TREATMENT OUTCOMES ACCORDING TO 1973 WHO PROTOCOL, (WHO, 1973)

- S or S/RI: In the extended test, the parasites are S if no asexual parasites are found by day 6 and parasites do not reappear by day 28. In the 7-day field test, the infection may be either S or resistant at RI (S/RI) level if no asexual parasites are found at day 6 and none are present on day 7. An S response cannot be distinguished from an RI response in the non-extended test, as the difference between the two responses depends on the presence or absence of recrudescence between day 8 and day 28.
- RI: In the extended test, parasites are resistant at the RI level if asexual parasites disappear but return within 28 days, reinfection having been excluded. In the 7-day field test, parasites are resistant at the RI level if asexual parasites disappear for at least 2 consecutive days but return and are present on day 7.
- RII: The parasites are resistant at the RII level if asexual parasitaemia does not clear but is reduced to 25% or less of the pre-test level during the first 48 h of treatment.
- RIII: The parasites are resistant at the RIII level if asexual parasitaemia is reduced by less than 75% during the first 48 h or if it continues to rise.

A1.2 Data entry

The importance of including in the database the many studies that did not follow the standard WHO protocol has been recognized. A number of changes to the WHO protocol (with varying degrees of justification) have been introduced in the field, including changes to the inclusion and exclusion criteria (age, history of fever, parasitaemia cut-off point) and the classification or analysis of data. While teams might sometimes have to adapt the protocol to the local epidemiological profile (e.g. changes to the inclusion and exclusion criteria that allow the inclusion of more cases as the number of cases of malaria at sentinel sites decreases), it is difficult to compare their results with those of studies that followed the standard protocol. All modifications, described below, are recorded in the database. When possible, all treatment outcomes are entered in the database according to standard methods for classifying outcomes.

MODIFICATIONS TO INCLUSION AND EXCLUSION CRITERIA

Targeted age and other groups

In all regions, the priority should be to measure treatment efficacy in young children (< 5 years) with clinical malaria. Even in populations with low levels of acquired immunity, young children often respond less favourably to antimalarial drugs than older children and adults. In countries where transmission is low or where young children are less exposed than adults to the risk of infection, preferential enrolment of children under 5 years of age has been difficult. Consequently, in order to obtain a large enough sample, many teams either extended the duration of enrolment or enrolled older children or adults. In practically all the countries of the Sahel and southern Africa, the maximum age at inclusion has been extended to 10 or 15 years or even to all age groups.

HIV infection is associated with an increased frequency of malarial episodes and higher parasitaemia. As immunity is a major factor in the therapeutic response, the immune suppression induced by HIV can compromise the outcome of antimalarial treatment. Chronic infections are an exclusion criterion for routine monitoring of therapeutic efficacy; however, because of the high prevalence of HIV positivity in areas in which malaria is endemic, clinical research is still needed for this group. Therapeutic efficacy studies have been performed in this patient population, either alone or in comparison with an HIV-negative control group.

During a first or second pregnancy, protective immunity against malaria tends to diminish, without disappearing completely. Together with changes in pharmacokinetics (apparent increase in the volume of distribution, with a resulting reduction in plasma drug concentration), this fall in immunity is responsible for a higher treatment failure rate among pregnant women than among other women of the same age. Like *Plasmodium*–HIV co-infection, pregnancy is an exclusion criterion. Clinical research on this group is important, however, as studies on therapeutic efficacy are necessary for appropriate implementation of intermittent preventive treatment within a national policy. Such studies are also important for determining the appropriate regimen, tolerance and adverse effects in pregnant women.

Severe, complicated malaria is a major exclusion criterion, and the appearance of signs of severe malaria after day 0 is considered to be a protocol violation. As such patients cannot take drugs orally, the efficacy of drugs administered by other routes has been evaluated in several studies. The results of these studies are difficult to interpret, as a considerable proportion of the patients die when their parasitaemia has completely disappeared.

Fever or history of fever in areas of high transmission

In 2001, it was agreed that patient enrolment in regions of high transmission should be based on the presence of fever at the time of admission. Similarly, determination of clinical treatment failure should be based on the presence of fever, as a history of fever alone is not adequate. It was argued that fever should be present at both the time of enrolment and the time of classification of outcome, in order to maintain consistency and to avoid including or classifying asymptomatic carriers as clinical failures. It was also recognized, however, that fever associated with malaria is not constant, that patients might have taken antipyretic drugs and that the absence of fever at the time of inclusion does not signify that the patient is not affected by malaria requiring treatment. In Africa, many teams prefer to use a history of fever as an inclusion criterion. Further, the enrolment of children with a history of fever could increase the number eligible for inclusion by 40%. None of the available studies show that the outcome was modified by inclusion of people with a history of fever in the previous 24 h instead of on admission.

In the 2009 WHO protocol for assessing antimalarial drug efficacy, either presentation of fever ≥ 37.5 °C or a history of fever in the previous 24 h is recommended. For consistency, however, whichever criterion is chosen, it should be applied throughout the study. In principle, there should be no problem in enrolling a sufficient number of febrile children in areas of high transmission. If there are difficulties of enrolment with use of these criteria, local transmission should be re-evaluated and the protocol adapted accordingly. In all cases, a history of fever should be limited to 24 h; there is no rationale for extending the history to 48 or 72 h.

Parasitaemia

At some sentinel sites, the minimum level of parasitaemia required for inclusion has been lowered in order to increase enrolment; e.g. in South America, a minimum parasitaemia limit of 250 per µl has been adopted. Use of a very low level of parasitaemia as an inclusion criterion presents two problems. First, in areas of high transmission, immune people are often asymptomatic carriers of low-grade parasitaemia, which can disappear spontaneously, thereby resulting in an overestimate of therapeutic success. Secondly, reading of microscope slides must be accurate in order to avoid errors in classifying early treatment failure on the basis of a comparison of parasitaemia on day 0 and on day 2 or 3. Adoption of a high minimum level (5000 per µl) in some studies resulted in the exclusion of a large number of patients, thus potentially introducing selection bias.

Raising the upper limit of parasitaemia allows for a 25% increase in the number of patients who can be included. A maximum level of 250 000 per µl has been used in areas of high transmission. Inclusion of patients with hyperparasitaemia (> 5%) should nevertheless be avoided on the grounds of patient safety, especially when the treatment is of uncertain efficacy or acts slowly. Furthermore, for some drugs, high parasitaemia is a risk factor for therapeutic failure.

Previous intake of an antimalarial drug

A recent history of antimalarial drug use or the presence of antimalarial drugs in the urine or blood is not an exclusion criterion in the WHO protocol. The protocol is not designed to evaluate the efficacy of drugs in the developmental phase. At many sentinel sites, previous treatment with antimalarial drugs is common in the target population (e.g. patients consulting in health centres for treatment of uncomplicated malaria), and exclusion of previously treated patients would mean that the sample was not representative. Further, self-reported histories of previous treatment are not always reliable: antimalarial drugs can be detected in the blood or urine of people who deny self-medication, or, conversely, negative results can be found for people who affirm that they have recently taken 'antimalarial treatment'. The exclusion of patients with a history of self-medication or recent antimalarial treatment might therefore reduce the number of patients who satisfy the inclusion criteria. Nonetheless, in a considerable number of studies, previous antimalarial drug intake was an exclusion criterion, particularly when the total dose of treatment had been administered. The main reason for exclusion is patient safety, as administration of the same or another treatment could have side-effects due to overdose or drug interactions. In addition, excluding such patients avoids sequential treatment, which might modify the final treatment outcome.

METHODS FOR CLASSIFYING TREATMENT OUTCOME

Duration of follow-up

Increasing numbers of reports of chloroquine resistance began to appear in the mid-1990s. The 1996 WHO protocol was a response designed to encourage simple, practical studies that could quickly determine whether chloroquine, amodiaquine and sulfadoxine–pyrimethamine were still effective in Africa. It was agreed that a 14-day follow-up period would be adequate for this purpose. At the time, national malaria control programmes did not have access to molecular biology laboratories that could perform the PCR tests needed to confirm whether treatment failure was due to recrudescence or reinfection. As access to these laboratories improved, the follow-up was increased to 28 days in the 2001 WHO protocol, with PCR correction recommended in high-transmission areas where there is an increased risk for reinfection. The 2009 WHO protocol maintains the 28-day follow-up as the minimum standard, with longer follow-up periods recommended for medicines with longer half-lives.

Management of parasitological failures

In 2001, the definition and management of parasitological failures without clinical signs differed between areas of high and low-to-moderate transmission. These differences reflected distinct regional programme priorities (clinical cure versus clinical and parasitological cure). In high-transmission areas, where people acquire underlying immunity during lifetime exposure, the presence of parasites without concurrent clinical symptoms is common. Therefore, rescue treatment was usually given only for clinical failures and not for asymptomatic parasitological failures in therapeutic efficacy studies. Only patients who had asymptomatic parasitaemia on the last day of the study were given rescue treatment and were classified as having late parasitological failure. In contrast, in low-to-moderate-transmission areas, where population immunity is low, parasitological and clinical failures were considered to be of equal weight, and rescue treatment

was always provided for both types of failure. In the 2009 WHO protocol, there are no differences in definition and management, and rescue treatment for patients with parasitological treatment failure is now recommended at all levels of malaria transmission.

Most of the studies included in the analysis were conducted according the 2001 WHO protocol, as few countries in high-transmission areas followed the 2009 WHO protocol. The differences between the two protocols are not, however, expected to have a strong influence on the overall results (Table A1.1). For example, in the 2001 protocol, if asymptomatic patients were found to be parasitaemic, they were observed until day 28, when they were given treatment and an outcome classification. Most patients either remain asymptomatic or progress to clinical failure; it is rare to see complete eradication of parasites without additional treatment. In the 2009 WHO protocol, there is no delay: patients are evaluated and treated on the day that parasitaemia is detected. Therefore, regardless of whether the 2001 or the 2009 WHO protocol is used, the total number of treatment failures, which is the main outcome used in the analysis, will be comparable. Only the proportion of late clinical failures and late parasitological failures may differ. Note that the classification for low-to-moderate-transmission areas is the same in 2001 and 2009.

Methods of analysis of the studies

Two types of analysis are typically used for antimalarial efficacy studies: per-protocol and intention-to-treat analyses. In the per-protocol analysis, only those patients who complete the entire study follow-up and have a clear outcome of either treatment success or failure are included. Those patients who do not complete follow-up, deviate from the study protocol or withdraw are excluded entirely. In the intention-to-treat analysis, all patients are included in the analysis, and the outcomes of all patients are designated as either a success or a failure. Patients who do not complete the study are generally classified as having treatment failure. This method is more suitable for comparative drug trials, in which patients are randomized, than for surveillance studies. In both per-protocol and intention-to-treat analyses, simple proportions or survival analysis can be used to measure outcomes.

In the modified version of the per-protocol method, survival analysis is used in order to include all patients in the analysis. Patients who do not complete the study are still included, as non-failures, but are censored on the last day of follow-up. Patients who withdraw or who are lost to follow-up before the end of the study are included up until the day of withdrawal or loss to follow-up. The outcomes of these patients are not considered failures or successes, and the study days that the patients contribute are retained as part of the survival analysis. The WHO protocol recommends either the original or the modified per-protocol method (referred to in the 2009 WHO protocol as Kaplan-Meier analysis). Both methods are accessible in Excel® programs developed by WHO. The per-protocol analysis is the more conservative choice, as it tends to result in an overestimate of the true treatment failure rate.

An error made in many studies with per-protocol analysis is that people who were lost to follow-up or withdrew from the study are retained in the analysis, which effectively results in an underestimate of the success rate. In these cases, before the results are entered into the WHO database, the rates are recalculated (i.e. patients lost to follow-up or excluded are removed from the denominator).

As stressed previously, the same study design and the same analytical methods must be used if results are to be compared over time. In this report, the results presented are based on per-protocol analyses, as this was the commonest type of analysis used in 2000–2010. In some studies, the treatment outcomes were calculated by both methods. This allowed an analysis of the difference between the results: the two methods showed a strong linear correlation (r^2 = 0.98, n = 199; Figure A1.1). The failure rates were higher with per-protocol analysis than with Kaplan-Meier analysis, but the difference remained small when the number of patients lost to follow-up or excluded was low. The mean difference in treatment outcomes was 0.9% (range, 0–22%).

TABLE A1.1. Comparison of 2001 and 2009 classifications of treatment outcome in high-transmission areas

2001 WHO PROTOCOL	2009 WHO PROTOCOL
Early treatment failure • danger signs or severe malaria on day 1, 2 or 3 in the presence of parasitaemia; • parasitaemia on day 2 higher than on day 0, irrespective of axillary temperature; • parasitaemia on day 3 with axillary temperature ≥ 37.5 °C; or • parasitaemia on day 3 ≥ 25% of count on day 0.	**Early treatment failure** • danger signs or severe malaria on day 1, 2 or 3 in the presence of parasitaemia; • parasitaemia on day 2 higher than on day 0, irrespective of axillary temperature; • parasitaemia on day 3 with axillary temperature ≥ 37.5 °C; or • parasitaemia on day 3 ≥ 25% of count on day 0.
Late clinical failure • danger signs or severe malaria in the presence of parasitaemia on any day between day 4 and day 14 (day 28) in patients who did not previously meet any of the criteria for early treatment failure; or • presence of parasitaemia on any day between day 4 and day 14 (day 28) with axillary temperature ≥ 37.5 °C in patients who did not previously meet any of the criteria for early treatment failure.	**Late clinical failure** • danger signs or severe malaria in the presence of parasitaemia on any day between day 4 and day 28 (day 42) in patients who did not previously meet any of the criteria for early treatment failure; or • presence of parasitaemia on any day between day 4 and day 28 (day 42) with axillary temperature ≥ 37.5 °C in patients who did not previously meet any of the criteria for early treatment failure.
Late parasitological failure • presence of parasitaemia on day 14 (day 28) with axillary temperature < 37.5 °C in patients who did not previously meet any of the criteria for early treatment failure or late clinical failure.	**Late parasitological failure** • presence of parasitaemia on any day between day 7 and day 28 (day 42) with axillary temperature < 37.5 °C in patients who did not previously meet any of the criteria for early treatment failure or late clinical failure.
Adequate clinical and parasitological response • absence of parasitaemia on day 14 (day 28), irrespective of axillary temperature, in patients who did not previously meet any of the criteria for early treatment failure, late clinical failure or late parasitological failure.	**Adequate clinical and parasitological response** • absence of parasitaemia on day 28 (day 42), irrespective of axillary temperature, in patients who did not previously meet any of the criteria for early treatment failure, late clinical failure or late parasitological failure.

Classification of reinfection and recrudescence

WHO recommends that molecular tests be performed in studies with a follow-up period of 28 days or longer, to distinguish between reinfection and recrudescence. PCR tests are recommended from day 7 of follow-up, the earliest that new infections have been detected. Variations in the interpretation of PCR results and subsequent data analysis can significantly modify the study results.

A common inconsistency is failure to exclude (or censor) from the analysis cases of reinfection by *P. falciparum* or the appearance of *P. vivax*. The WHO protocol recommends excluding or censoring these cases because in both situations rescue treatment is administered, which is unrelated to the infection for which the patient was enrolled and which could mask a true recrudescence that cannot yet be detected by PCR. These patients, who may represent up to 50% or more of late treatment failures in areas of high transmission, are not excluded (or censored), probably because the number of patients remaining for analysis would be markedly reduced. In some studies, cases of reinfection were even considered treatment successes, resulting in an overestimate of the treatment success rate. Similarly, patients should be excluded if their PCR result is missing or does not clearly distinguish a reinfection from a recrudescence.

In several studies, failures that were not confirmed by PCR tests or for which the result was indeterminate were classified as treatment successes or as late treatment failures on the basis of mathematical calculations. In order to standardize methods, to allow meaningful comparisons, these types of inconsistencies are rectified, when possible, before the data are entered into the database. In areas of low-to-moderate transmission, however, PCR correction of results was not systematic until 2009, and this may have led to overestimates of the true treatment failure rates.

FIGURE A1.1. Correlation between per-protocol and Kaplan-Meier analyses

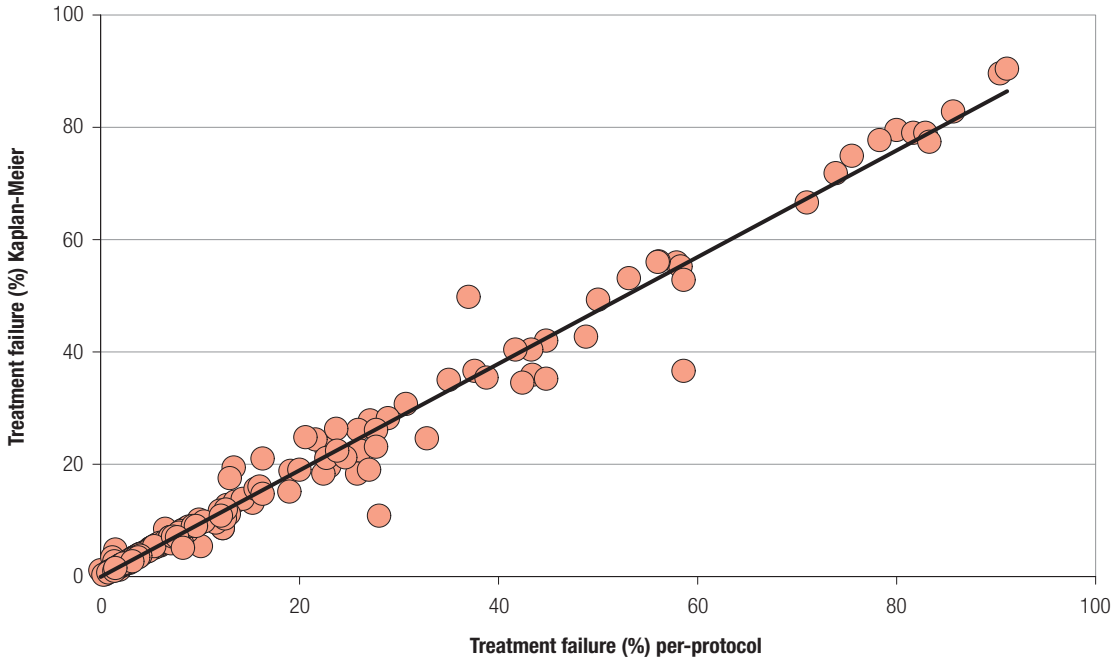

TABLE A1.2. Efficacy of antimalarial drugs against *P. falciparum* by WHO region and country, expressed as percentage of treatment failure, after a minimum 28-day follow-up*

	STUDY YEARS	NUMBER OF STUDIES	MEDIAN	MINIMUM	MAXIMUM
WHO AFRICAN REGION					
Angola					
Amodiaquine	2002–2003	2	20.4	19.1	21.6
Artemether–lumefantrine	2004–2004	2	1.2	0.0	2.3
Artesunate–amodiaquine	2003–2004	3	1.2	0.0	3.3
Artesunate–sulfadoxine–pyrimethamine	2003–2003	1	1.2	1.2	1.2
Chloroquine	2002–2002	1	85.7	85.7	85.7
Sulfadoxine–pyrimethamine	2002–2003	2	33.0	27.1	38.8
Benin					
Artemether–lumefantrine	2005–2007	4	0.8	0.0	6.5
Artesunate–amodiaquine	2007–2007	1	0.0	0.0	0.0
Artesunate–sulfadoxine–pyrimethamine	2003–2005	1	5.6	5.6	5.6
Chloroquine	2002–2005	6	35.5	15.0	73.9
Mefloquine	2005–2005	1	2.6	2.6	2.6
Sulfadoxine–pyrimethamine	2002–2007	8	35.7	3.3	71.7
Botswana					
Sulfadoxine–pyrimethamine	2006–2006	3	24.6	12.2	30.1
Burkina Faso					
Amodiaquine	2004–2005	7	10.9	2.6	63.9
Amodiaquine–sulfadoxine–pyrimethamine	2004–2006	3	2.1	0.5	3.9
Artemether–lumefantrine	2005–2007	4	4.3	1.9	12.3
Artesunate–amodiaquine	2004–2007	5	4.9	3.9	21.5
Artesunate–mefloquine	2007–2008	1	0.0	0.0	0.0
Artesunate–sulfalene–pyrimethamine	2004–2004	1	2.8	2.8	2.8
Dihydroartemisinin–piperaquine	2005–2006	2	1.9	1.5	2.2
Sulfadoxine–pyrimethamine	2003–2004	6	6.1	0.0	12.0
Burundi					
Artesunate–amodiaquine	2005–2006	2	5.2	2.9	7.5
Cameroon					
Amodiaquine	2004–2006	4	20.3	10.7	28.8
Amodiaquine–sulfadoxine–pyrimethamine	2001–2006	5	18.1	0.0	23.8
Artemether–lumefantrine	2006–2009	6	0.7	0.0	2.3
Artesunate–amodiaquine	2005–2009	9	3.7	0.0	8.7
Artesunate–mefloquine	2006–2009	2	1.8	0.0	3.5
Artesunate–sulfadoxine–pyrimethamine	2005–2007	2	3.6	1.4	5.7
Artesunate–sulfalene–pyrimethamine	2006–2007	1	1.2	1.2	1.2
Dihydroartemisinin–piperaquine	2007–2007	1	2.3	2.3	2.3
Sulfadoxine–pyrimethamine	2004–2006	3	32.5	29.9	37.5

* If follow-up to day 28 was not available, the alternate follow-up day is given between brackets.

	STUDY YEARS	NUMBER OF STUDIES	MEDIAN	MINIMUM	MAXIMUM
Central African Republic					
Artesunate 7-day treatment	2004–2004	1	5.6	5.6	5.6
Chad					
Amodiaquine	2002–2003	2	4.4	2.2	6.5
Chloroquine	2002–2003	2	28.3	23.7	32.8
Sulfadoxine–pyrimethamine	2002–2003	2	10.3	4.3	16.3
Comoros					
Artemether–lumefantrine	2004–2007	9	0.0	0.0	3.2
Congo					
Amodiaquine	2004–2004	1	34.8	34.8	34.8
Amodiaquine–sulfadoxine–pyrimethamine	2004–2005	1	14.3	14.3	14.3
Artemether–lumefantrine	2004–2006	2	1.6	0.0	3.2
Artesunate–amodiaquine	2004–2004	1	1.5	1.5	1.5
Artesunate–sulfadoxine–pyrimethamine	2004–2004	1	9.9	9.9	9.9
Chloroquine	2003–2003	1	95.1	95.1	95.1
Sulfadoxine–pyrimethamine	2003–2004	1	31.2	31.2	31.2
Côte d'Ivoire					
Artemether–lumefantrine	2005–2009	4	2.1	0.0	7.4
Artesunate–amodiaquine	2008–2009	2	0.0	0.0	0.0
Artesunate–sulfalene–pyrimethamine	2005–2005	2	0.0	0.0	0.0
Democratic Republic of the Congo					
Amodiaquine	2003–2004	1	25.8	25.8	25.8
Amodiaquine–sulfadoxine–pyrimethamine	2005–2005	1	5.4	5.4	5.4
Artemether–lumefantrine	2005–2008	6	2.4	0.0	9.2
Artesunate–amodiaquine	2003–2005	8	6.2	0.0	19.0
Artesunate–pyronaridine	2007–2008	1	0.5	0.5	0.5
Artesunate–sulfadoxine–pyrimethamine	2003–2004	3	19.7	0.0	32.8
Sulfadoxine–pyrimethamine	2003–2004	2	33.2	23.0	43.4
Equatorial Guinea					
Artesunate–amodiaquine	2006–2006	1	3.3	3.3	3.3
Eritrea					
Artemether–lumefantrine	2007–2007	3	0.0	0.0	0.0
Artesunate–amodiaquine	2006–2009	8	4.1	1.5	12.5
Chloroquine–sulfadoxine–pyrimethamine	2006–2006	1	50.0	50.0	50.0
Ethiopia					
Artemether–lumefantrine	2003–2009	9	0.0	0.0	7.5
Atovaquone–proguanil	2006–2006	1	6.7	6.7	6.7
Quinine	2006–2006	1	10.0	10.0	10.0
Sulfadoxine–pyrimethamine	2003–2003	11	71.1	52.8	85.7

	STUDY YEARS	NUMBER OF STUDIES	MEDIAN	MINIMUM	MAXIMUM
Gabon					
Amodiaquine	2000–2005	2	33.5	33.0	33.9
Artesunate 5-day treatment	2002–2004	1	9.5	9.5	9.5
Artesunate–amodiaquine	2004–2005	1	13.8	13.8	13.8
Artesunate–mefloquine	2005–2006	2	0.0	0.0	0.0
Artesunate–pyronaridine	2006–2006	1	0.0	0.0	0.0
Sulfadoxine–pyrimethamine	2000–2006	3	13.5	6.1	14.0
Gambia					
Artemether–lumefantrine	2002–2008	2	2.0	0.0	3.9
Artesunate–pyronaridine	2007–2008	1	0.0	0.0	0.0
Chloroquine	2001–2001	1	30.2	30.2	30.2
Chloroquine–sulfadoxine–pyrimethamine	2001–2002	2	6.4	3.9	8.9
Sulfadoxine–pyrimethamine	2001–2001	1	6.1	6.1	6.1
Ghana					
Amodiaquine–sulfadoxine–pyrimethamine	2002–2002	1	6.0	6.0	6.0
Artemether–lumefantrine	2003–2007	5	4.0	1.7	13.8
Artesunate–amodiaquine	2003–2006	4	4.3	0.0	14.0
Artesunate–sulfadoxine–pyrimethamine	2002–2002	1	6.1	6.1	6.1
Chloroquine	2003–2003	1	75.0	75.0	75.0
Sulfadoxine–pyrimethamine	2002–2002	1	28.7	28.7	28.7
Guinea					
Artesunate–amodiaquine	2004–2004	1	1.0	1.0	1.0
Artesunate–sulfadoxine–pyrimethamine	2004–2004	1	1.0	1.0	1.0
Guinea-Bissau					
Amodiaquine	2001–2004	1	6.0	6.0	6.0
Chloroquine	2001–2004	1	20.0	20.0	20.0
Kenya					
Amodiaquine	2006–2006	1	20.2	20.2	20.2
Amodiaquine–sulfadoxine–pyrimethamine	2004–2004	1	9.0	9.0	9.0
Artemether–lumefantrine	2002–2008	12	2.7	0.0	6.6
Artesunate–amodiaquine	2004–2007	3	12.3	9.1	13.3
Artesunate–pyronaridine	2007–2008	1	0.0	0.0	0.0
Artesunate–sulfadoxine–pyrimethamine	2003–2004	1	15.0	15.0	15.0
Dihydroartemisinin–piperaquine	2005–2007	2	4.2	0.0	8.3
Sulfadoxine–pyrimethamine	2002–2004	2	31.7	8.0	55.3
Liberia					
Amodiaquine	2001–2001	1	22.9	22.9	22.9
Artemether–lumefantrine	2007–2007	2	0.0	0.0	0.0
Artesunate–amodiaquine	2007–2007	2	0.0	0.0	0.0
Sulfadoxine–pyrimethamine	2000–2000	1	69.7	69.7	69.7

	STUDY YEARS	NUMBER OF STUDIES	MEDIAN	MINIMUM	MAXIMUM
Madagascar					
Amodiaquine	2006–2007	8	0.9	0.0	6.4
Amodiaquine–sulfadoxine–pyrimethamine	2006–2006	1	3.9	3.9	3.9
Artemether–lumefantrine	2006–2006	1	1.7	1.7	1.7
Artesunate–amodiaquine	2006–2007	10	0.0	0.0	8.7
Chloroquine	2006–2006	6	51.4	22.4	71.0
Sulfadoxine–pyrimethamine	2003–2007	9	2.7	0.0	12.9
Malawi					
Amodiaquine–sulfadoxine–pyrimethamine	2003–2005	2	4.3	3.0	5.5
Artemether–lumefantrine	2005–2005	1	7.1	7.1	7.1
Artesunate–amodiaquine	2005–2005	2	1.8	0.0	3.6
Artesunate–sulfadoxine–pyrimethamine	2003–2005	1	26.0	26.0	26.0
Chloroquine	2005–2005	1	1.3	1.3	1.3
Chloroquine–sulfadoxine–pyrimethamine	2003–2005	1	14.0	14.0	14.0
Sulfadoxine–pyrimethamine	2003–2005	3	74.0	68.2	81.7
Mali					
Amodiaquine	2002–2004	2	13.2	11.5	14.8
Amodiaquine–sulfadoxine–pyrimethamine	2005–2006	1	0.8	0.8	0.8
Artemether–lumefantrine	2004–2008	6	3.0	0.0	6.0
Artesunate 5-day treatment	2002–2004	1	3.5	3.5	3.5
Artesunate–amodiaquine	2002–2006	4	2.0	0.0	7.6
Artesunate–pyronaridine	2007–2008	1	0.0	0.0	0.0
Artesunate–sulfadoxine–pyrimethamine	2002–2006	2	1.6	0.0	3.2
Artesunate–sulfalene–pyrimethamine	2003–2007	2	0.0	0.0	0.0
Chloroquine	2002–2004	3	36.8	24.5	90.4
Dihydroartemisinin 5-day treatment	2001–2003	1	7.9	7.9	7.9
Sulfadoxine–pyrimethamine	2002–2004	3	3.4	2.0	7.0
Mozambique					
Artemether–lumefantrine	2005–2008	4	1.6	0.0	3.1
Artesunate–pyronaridine	2007–2008	1	0.0	0.0	0.0
Artesunate–sulfadoxine–pyrimethamine [42 d]	2003–2005	1	2.3	2.3	2.3
Dihydroartemisinin–piperaquine	2005–2006	1	6.9	6.9	6.9
Sulfadoxine–pyrimethamine [42 d]	2002–2005	3	25.0	11.6	27.0
Namibia					
Chloroquine	2003–2003	3	67.7	55.5	78.7
Sulfadoxine–pyrimethamine	2003–2003	3	33.4	9.1	36.8
Niger					
Artemether–lumefantrine	2006–2006	1	4.4	4.4	4.4
Chloroquine	2005–2005	1	36.8	36.8	36.8
Sulfadoxine–pyrimethamine	2005–2006	2	16.7	16.6	16.7

	STUDY YEARS	NUMBER OF STUDIES	MEDIAN	MINIMUM	MAXIMUM
Nigeria					
Amodiaquine	2000–2005	3	8.5	4.8	12.9
Amodiaquine–sulfadoxine–pyrimethamine	2000–2006	3	0.0	0.0	8.3
Amodiaquine–sulfalene–pyrimethamine [42 d]	2005–2006	1	1.1	1.1	1.1
Artemether–lumefantrine	2002–2007	5	0.0	0.0	2.0
Artesunate–amodiaquine	2004–2006	5	0.0	0.0	7.8
Artesunate–mefloquine	2007–2008	1	2.9	2.9	2.9
Chloroquine	2002–2005	2	43.3	37.9	48.6
Chloroquine–sulfadoxine–pyrimethamine	2004–2004	1	10.0	10.0	10.0
Mefloquine	2007–2008	1	8.8	8.8	8.8
Sulfadoxine–pyrimethamine	2003–2004	3	25.0	20.6	27.0
Rwanda					
Amodiaquine	2001–2002	6	19.4	14.3	30.0
Amodiaquine–sulfadoxine–pyrimethamine	2001–2006	10	14.5	2.0	68.1
Artemether–lumefantrine	2004–2007	3	1.5	0.0	6.9
Artesunate–amodiaquine	2002–2004	6	6.2	4.7	22.5
Artesunate–sulfadoxine–pyrimethamine	2001–2006	4	13.1	8.5	33.3
Artesunate–sulfalene–pyrimethamine	2005–2007	2	2.5	1.1	3.9
Dihydroartemisinin–piperaquine	2003–2004	3	1.4	1.2	12.5
Senegal					
Amodiaquine–sulfadoxine–pyrimethamine	2003–2003	1	0.0	0.0	0.0
Artemether–lumefantrine	2002–2008	6	0.9	0.0	3.2
Artesunate–amodiaquine	2002–2008	7	0.0	0.0	0.5
Artesunate–mefloquine	2008–2008	1	0.0	0.0	0.0
Artesunate–pyronaridine	2007–2008	1	0.0	0.0	0.0
Sierra Leone					
Amodiaquine	2002–2003	5	15.6	7.1	42.4
Artesunate–amodiaquine	2004–2004	1	27.0	27.0	27.0
Chloroquine	2002–2003	3	83.3	58.6	91.1
Sulfadoxine–pyrimethamine	2002–2003	3	27.7	24.6	48.8
South Africa					
Artemether–lumefantrine	2002–2007	3	0.0	0.0	5.2
Artesunate–sulfadoxine–pyrimethamine [42 d]	2004–2004	1	1.1	1.1	1.1
Sulfadoxine–pyrimethamine [42 d]	2000–2002	3	10.0	6.4	88.3
Togo					
Artemether–lumefantrine	2005–2008	6	1.5	0.0	4.4
Artesunate–amodiaquine	2005–2008	6	0.0	0.0	6.1

	STUDY YEARS	NUMBER OF STUDIES	MEDIAN	MINIMUM	MAXIMUM
Uganda					
Amodiaquine	2002–2002	1	20.6	20.6	20.6
Amodiaquine–sulfadoxine–pyrimethamine	2001–2008	12	14.6	7.0	38.0
Artemether–lumefantrine	2002–2008	8	2.3	0.0	8.9
Artesunate–amodiaquine	2002–2008	9	5.2	3.8	12.0
Chloroquine–sulfadoxine–pyrimethamine	2001–2004	11	41.0	22.0	73.0
Dihydroartemisinin–piperaquine	2005–2008	4	1.4	0.3	5.5
Sulfadoxine–pyrimethamine	2001–2002	2	47.3	37.0	57.6
United Republic of Tanzania					
Mainland					
Amodiaquine	2004–2005	2	46.7	40.4	53.0
Artemether–lumefantrine	2002–2008	8	2.9	0.0	8.6
Artesunate–amodiaquine	2004–2007	2	4.4	2.0	6.8
Artesunate–sulfadoxine–pyrimethamine	2006–2006	2	5.6	4.7	6.4
Sulfadoxine–pyrimethamine	2003–2006	7	43.3	25.5	82.2
Zanzibar					
Artemether–lumefantrine	2002–2007	3	0.0	0.0	2.7
Artesunate–amodiaquine [42 d]	2002–2005	2	12.1	10.8	13.4
Zambia					
Artemether–lumefantrine	2004–2006	12	0.0	0.0	6.7
Artesunate–sulfadoxine–pyrimethamine	2002–2004	3	17.5	3.5	26.5
Dihydroartemisinin–piperaquine	2005–2006	1	4.7	4.7	4.7
Sulfadoxine–pyrimethamine	2004–2005	3	33.9	23.2	34.4
Zimbabwe					
Artemether–lumefantrine	2007–2007	3	0.0	0.0	1.9
WHO REGION OF THE AMERICAS					
Brazil					
Artemether–lumefantrine	2005–2007	2	0.0	0.0	0.0
Artesunate–mefloquine	2005–2007	3	0.0	0.0	0.0
Mefloquine	2000–2000	1	2.4	2.4	2.4
Colombia					
Amodiaquine	2000–2004	4	28.8	2.8	31.2
Amodiaquine–sulfadoxine–pyrimethamine	2001–2006	2	8.3	5.7	10.8
Artesunate–amodiaquine	2000–2006	2	0.0	0.0	0.0
Artesunate–mefloquine	2007–2008	1	0.0	0.0	0.0
Artesunate–sulfadoxine–pyrimethamine	2002–2006	3	2.1	2.0	3.4
Sulfadoxine–pyrimethamine	2001–2005	2	10.7	5.3	16.1

	STUDY YEARS	NUMBER OF STUDIES	MEDIAN	MINIMUM	MAXIMUM
Ecuador					
Amodiaquine	2004–2004	1	46.7	46.7	46.7
Amodiaquine–sulfadoxine–pyrimethamine	2004–2004	1	0.0	0.0	0.0
Artemether–lumefantrine	2005–2006	1	0.0	0.0	0.0
Artesunate–sulfadoxine–pyrimethamine	2003–2004	2	0.0	0.0	0.0
Chloroquine–sulfadoxine–pyrimethamine	2003–2003	1	0.0	0.0	0.0
Sulfadoxine–pyrimethamine	2001–2003	4	2.0	0.0	17.1
Guyana					
Artemether–lumefantrine	2004–2008	2	1.6	0.0	3.2
Artesunate–mefloquine	2004–2005	1	1.2	1.2	1.2
Mefloquine	2004–2005	1	3.6	3.6	3.6
Honduras					
Chloroquine	2008–2009	1	0.0	0.0	0.0
Nicaragua					
Chloroquine	2005–2006	1	0.0	0.0	0.0
Sulfadoxine–pyrimethamine	2005–2006	1	0.0	0.0	0.0
Peru					
Artesunate–mefloquine	2003–2006	3	0.0	0.0	0.0
Artesunate–sulfadoxine–pyrimethamine	2000–2000	1	1.1	1.1	1.1
Dihydroartemisinin–piperaquine [63 d]	2003–2005	1	1.8	1.8	1.8
Sulfadoxine–pyrimethamine	2000–2002	2	9.5	3.3	15.6
Suriname					
Artemether–lumefantrine	2003–2006	3	2.0	1.9	4.7
Artesunate–mefloquine [35 d]	2002–2003	2	4.1	2.4	5.8
Mefloquine [35 d]	2002–2002	1	7.3	7.3	7.3
Venezuela (Bolivarian Republic of)					
Artemether–lumefantrine	2004–2005	1	0.0	0.0	0.0
Artesunate–mefloquine	2004–2005	1	0.0	0.0	0.0
Quinine	2002–2003	3	20.0	9.6	22.2
WHO SOUTH-EAST ASIA REGION					
Bangladesh					
Artemether–lumefantrine [42 d]	2003–2007	3	2.9	0.0	5.7
Artesunate–mefloquine	2003–2003	1	0.9	0.9	0.9
Chloroquine	2004–2004	1	57.7	57.7	57.7
Chloroquine–sulfadoxine–pyrimethamine	2002–2003	6	28.3	12.9	33.0
Bhutan					
Artesunate–doxycycline	2000–2005	11	5.0	0.0	22.4

	STUDY YEARS	NUMBER OF STUDIES	MEDIAN	MINIMUM	MAXIMUM
India					
Artemether–lumefantrine	2007–2008	3	0.0	0.0	1.4
Artesunate–sulfadoxine–pyrimethamine	2005–2007	9	0.0	0.0	4.0
Chloroquine	2001–2008	85	37.5	0.0	100.0
Sulfadoxine–pyrimethamine	2001–2007	22	13.6	0.0	56.7
Indonesia					
Artemether–lumefantrine	2004–2008	3	3.1	0.0	7.7
Artesunate–amodiaquine	2003–2006	8	8.8	0.0	24.1
Chloroquine	2001–2002	3	72.5	69.1	81.1
Chloroquine–sulfadoxine–pyrimethamine	2001–2004	2	6.2	6.2	6.2
Dihydroartemisinin–piperaquine [42 d]	2004–2008	3	4.1	2.7	4.8
Sulfadoxine–pyrimethamine	2001–2005	6	10.0	4.7	59.1
Myanmar					
Artemether–lumefantrine	2004–2009	15	2.5	0.0	8.3
Artemisinin–piperaquine	2008–2008	1	6.0	6.0	6.0
Artesunate–amodiaquine	2004–2007	13	3.4	0.0	19.2
Artesunate–mefloquine	2000–2006	9	1.8	0.0	8.9
Dihydroartemisinin 5-day treatment	2003–2003	1	10.5	10.5	10.5
Dihydroartemisinin 7-day treatment	2003–2003	1	13.1	13.1	13.1
Dihydroartemisinin–piperaquine	2003–2009	4	2.9	0.0	5.0
Nepal					
Artemether–lumefantrine	2005–2008	2	0.0	0.0	0.0
Sulfadoxine–pyrimethamine	2003–2005	3	19.3	12.1	22.0
Sri Lanka					
Artesunate–sulfadoxine–pyrimethamine	2000–2000	1	0.0	0.0	0.0
Chloroquine	2000–2004	4	40.8	10.0	100.0
Sulfadoxine–pyrimethamine	2002–2002	1	0.0	0.0	0.0
Thailand					
Artemether–lumefantrine	2001–2006	3	0.0	0.0	0.9
Artemisinin–piperaquine	2005–2007	2	1.7	1.6	1.8
Artesunate–mefloquine	2001–2009	20	0.5	0.0	10.4
Atovaquone–proguanil	2004–2005	1	2.2	2.2	2.2
Dihydroartemisinin–piperaquine [63 d]	2002–2004	4	1.3	0.0	3.9
Timor-Leste					
Chloroquine	2000–2000	1	63.7	63.7	63.7
Sulfadoxine–pyrimethamine	2001–2001	1	7.9	7.9	7.9

	STUDY YEARS	NUMBER OF STUDIES	MEDIAN	MINIMUM	MAXIMUM
WHO EUROPEAN REGION					
Tajikistan					
Artesunate–sulfadoxine–pyrimethamine	2004–2004	1	0.0	0.0	0.0
Chloroquine	2002–2002	1	56.0	56.0	56.0
Chloroquine–sulfadoxine–pyrimethamine	2003–2003	1	2.1	2.1	2.1
Quinine	2003–2003	1	0.0	0.0	0.0
Sulfadoxine–pyrimethamine	2002–2002	1	16.0	16.0	16.0
WHO EASTERN MEDITERRANEAN REGION					
Afghanistan					
Amodiaquine	2003–2004	1	37.6	37.6	37.6
Amodiaquine–sulfadoxine–pyrimethamine	2003–2004	2	2.0	1.0	3.0
Artesunate–amodiaquine [42 d]	2002–2003	1	28.4	28.4	28.4
Artesunate–sulfadoxine–pyrimethamine	2004–2006	5	0.0	0.0	0.0
Chloroquine	2002–2002	1	89.5	89.5	89.5
Chloroquine–sulfadoxine–pyrimethamine	2004–2004	2	0.0	0.0	0.0
Sulfadoxine–pyrimethamine	2002–2003	3	8.7	4.0	22.7
Iran (Islamic Republic of)					
Artesunate–sulfadoxine–pyrimethamine	2005–2007	4	0.0	0.0	0.0
Chloroquine	2000–2004	4	73.6	61.0	78.3
Chloroquine–sulfadoxine–pyrimethamine	2005–2007	5	1.9	0.0	9.1
Sulfadoxine–pyrimethamine	2001–2001	1	0.0	0.0	0.0
Pakistan					
Amodiaquine	2004–2005	1	53.1	53.1	53.1
Artesunate–sulfadoxine–pyrimethamine	2004–2008	6	0.0	0.0	3.2
Chloroquine	2000–2005	4	38.4	20.0	82.9
Quinine–doxycycline	2003–2004	1	0.0	0.0	0.0
Sulfadoxine–pyrimethamine	2004–2005	1	56.1	56.1	56.1
Somalia					
Artesunate–amodiaquine	2006–2006	2	2.1	1.9	2.2
Artesunate–sulfadoxine–pyrimethamine	2004–2006	3	1.0	0.0	5.1
Sudan					
High-transmission area					
Amodiaquine	2001–2005	2	38.6	20.0	57.1
Artemether–lumefantrine	2004–2004	1	2.8	2.8	2.8
Artesunate–amodiaquine	2003–2005	2	3.1	1.0	5.1
Artesunate–sulfadoxine–pyrimethamine	2003–2004	2	0.5	0.0	0.9
Chloroquine	2001–2002	2	100.0	100.0	100.0
Sulfadoxine–pyrimethamine	2001–2002	2	43.6	17.2	69.9

	STUDY YEARS	NUMBER OF STUDIES	MEDIAN	MINIMUM	MAXIMUM
Low-to-moderate-transmission area					
Artemether–lumefantrine	2004–2008	8	0.0	0.0	8.9
Artesunate–amodiaquine	2003–2005	2	6.1	5.0	7.2
Artesunate–sulfadoxine–pyrimethamine	2003–2008	12	1.5	0.0	9.7
Artesunate–sulfalene–pyrimethamine	2005–2007	2	1.5	0.0	2.9
Chloroquine	2003–2003	1	51.5	51.5	51.5
Chloroquine–sulfadoxine–pyrimethamine	2003–2003	4	10.2	2.5	36.6
Quinine	2002–2003	2	6.5	6.3	6.7
Sulfadoxine–pyrimethamine	2001–2003	7	7.6	2.0	31.7
Yemen					
Amodiaquine	2004–2004	1	44.2	44.2	44.2
Artemether–lumefantrine	2007–2007	1	0.0	0.0	0.0
Artesunate–amodiaquine	2004–2004	1	18.5	18.5	18.5
Artesunate–sulfadoxine–pyrimethamine	2007–2007	2	0.8	0.0	1.5
Sulfadoxine–pyrimethamine	2004–2005	3	0.0	0.0	5.0
WHO WESTERN PACIFIC REGION					
Cambodia					
Artemether–lumefantrine	2001–2004	4	21.3	13.5	28.9
Artesunate 7-day treatment	2006–2009	3	6.7	5.2	8.3
Artesunate–mefloquine	2001–2010	26	2.4	0.0	14.3
Atovaquone–proguanil	2008–2009	1	0.0	0.0	0.0
Dihydroartemisinin–piperaquine	2008–2010	5	1.3	0.0	7.9
Quinine–tetracycline	2006–2007	1	0.0	0.0	0.0
China					
Artemether–lumefantrine	2004–2005	1	0.0	0.0	0.0
Artesunate 5-day treatment	2002–2002	1	20.6	20.6	20.6
Artesunate 7-day treatment	2009–2009	1	0.0	0.0	0.0
Chloroquine	2005–2006	1	40.6	40.6	40.6
Dihydroartemisinin 7-day treatment	2001–2002	1	2.9	2.9	2.9
Dihydroartemisinin–piperaquine	2004–2009	2	0.0	0.0	0.0
Lao People's Democratic Republic					
Artemether–lumefantrine	2002–2006	4	1.6	0.0	6.3
Artesunate–mefloquine	2002–2004	3	0.0	0.0	0.0
Chloroquine	2001–2001	1	31.3	31.3	31.3
Chloroquine–sulfadoxine–pyrimethamine	2002–2003	1	6.9	6.9	6.9
Dihydroartemisinin–piperaquine	2004–2004	1	0.0	0.0	0.0
Sulfadoxine–pyrimethamine	2000–2003	3	24.0	18.7	33.0

	STUDY YEARS	NUMBER OF STUDIES	MEDIAN	MINIMUM	MAXIMUM
Malaysia					
Chloroquine	2003–2004	2	61.8	45.2	78.4
Chloroquine–sulfadoxine–pyrimethamine	2000–2004	4	41.4	25.0	62.5
Sulfadoxine–pyrimethamine	2003–2004	2	19.4	16.6	22.2
Papua New Guinea					
Amodiaquine–sulfadoxine–pyrimethamine	2002–2005	6	19.6	10.3	28.8
Artemether–lumefantrine	2006–2007	1	2.7	2.7	2.7
Artesunate–sulfadoxine–pyrimethamine	2006–2007	1	10.0	10.0	10.0
Chloroquine–sulfadoxine–pyrimethamine	2004–2007	4	16.8	13.3	22.2
Dihydroartemisinin–piperaquine	2006–2007	1	9.9	9.9	9.9
Philippines					
Artemether–lumefantrine	2003–2009	9	0.0	0.0	5.6
Artesunate–pyronaridine	2007–2008	1	0.0	0.0	0.0
Chloroquine–sulfadoxine–pyrimethamine	2001–2007	9	9.3	0.0	20.5
Sulfadoxine–pyrimethamine	2000–2001	5	42.6	8.5	60.6
Solomon Islands					
Artemether–lumefantrine	2008–2008	1	0.0	0.0	0.0
Chloroquine–sulfadoxine–pyrimethamine	2007–2007	2	17.9	11.6	24.2
Vanuatu					
Chloroquine–sulfadoxine–pyrimethamine	2001–2005	5	8.5	0.0	16.7
Viet Nam					
Artemether 5-day treatment	2003–2003	1	0.0	0.0	0.0
Artemether–lumefantrine	2001–2001	1	2.2	2.2	2.2
Artesunate 5-day treatment	2000–2005	3	12.5	11.9	17.5
Artesunate 7-day treatment	2002–2009	11	3.2	0.0	14.6
Artesunate–amodiaquine	2006–2007	1	2.1	2.1	2.1
Artesunate–mefloquine	2001–2008	3	0.0	0.0	1.3
Atovaquone–proguanil	2001–2002	1	5.2	5.2	5.2
Chloroquine	2000–2007	8	19.8	0.0	71.9
Dihydroartemisinin 5-day treatment	2002–2003	1	26.7	26.7	26.7
Dihydroartemisinin–piperaquine	2001–2010	14	0.0	0.0	6.1
Sulfadoxine–pyrimethamine	2002–2003	2	26.8	14.9	38.7

TABLE A1.3. Efficacy of antimalarial drugs against *P. falciparum* by drug, expressed as percentage of treatment failure, after a minimum 28-day follow-up*

	STUDY YEARS	NUMBER OF STUDIES	MEDIAN	MINIMUM	MAXIMUM
AMODIAQUINE					
WHO African Region					
Angola	2002–2003	2	20.4	19.1	21.6
Burkina Faso	2004–2005	7	10.9	2.6	63.9
Cameroon	2004–2006	4	20.3	10.7	28.8
Chad	2002–2003	2	4.4	2.2	6.5
Congo	2004–2004	1	34.8	34.8	34.8
Democratic Republic of the Congo	2003–2004	1	25.8	25.8	25.8
Gabon	2000–2005	2	33.5	33.0	33.9
Guinea-Bissau	2001–2004	1	6.0	6.0	6.0
Kenya	2006–2006	1	20.2	20.2	20.2
Liberia	2001–2001	1	22.9	22.9	22.9
Madagascar	2006–2007	8	0.9	0.0	6.4
Mali	2002–2004	2	13.2	11.5	14.8
Nigeria	2000–2005	3	8.5	4.8	12.9
Rwanda	2001–2002	6	19.4	14.3	30.0
Sierra Leone	2002–2003	5	15.6	7.1	42.4
Uganda	2002–2002	1	20.6	20.6	20.6
United Republic of Tanzania					
Mainland	2004–2005	2	46.7	40.4	53.0
WHO Region of the Americas					
Colombia	2000–2004	4	28.8	2.8	31.2
Ecuador	2004–2004	1	46.7	46.7	46.7
WHO Eastern Mediterranean Region					
Afghanistan	2003–2004	1	37.6	37.6	37.6
Pakistan	2004–2005	1	53.1	53.1	53.1
Sudan					
High-transmission area	2001–2004	2	38.6	20.0	57.1
Yemen	2004–2004	1	44.2	44.2	44.2
AMODIAQUINE–SULFADOXINE–PYRIMETHAMINE					
WHO African Region					
Burkina Faso	2004–2006	3	2.1	0.5	3.9
Cameroon	2001–2006	5	18.1	0.0	23.8
Congo	2004–2005	1	14.3	14.3	14.3
Democratic Republic of the Congo	2005–2005	1	5.4	5.4	5.4
Ghana	2002–2002	1	6.0	6.0	6.0

* If follow-up to day 28 was not available, the alternate follow-up day is given between brackets.

	STUDY YEARS	NUMBER OF STUDIES	MEDIAN	MINIMUM	MAXIMUM
Kenya	2004–2004	1	9.0	9.0	9.0
Madagascar	2006–2006	1	3.9	3.9	3.9
Malawi	2003–2005	2	4.3	3.0	5.5
Mali	2005–2006	1	0.8	0.8	0.8
Nigeria	2000–2006	3	0.0	0.0	8.3
Rwanda	2001–2006	10	14.5	2.0	68.1
Senegal	2003–2003	1	0.0	0.0	0.0
Uganda	2001–2008	12	14.6	7.0	38.0
WHO Region of the Americas					
Colombia	2001–2006	2	8.3	5.7	10.8
Ecuador	2004–2004	1	0.0	0.0	0.0
WHO Eastern Mediterranean Region					
Afghanistan	2003–2004	2	2.0	1.0	3.0
WHO Western Pacific Region					
Papua New Guinea	2002–2005	6	19.6	10.3	28.8
AMODIAQUINE–SULFALENE–PYRIMETHAMINE					
WHO African Region					
Nigeria [42 d]	2005–2006	1	1.1	1.1	1.1
ARTEMETHER 5-DAY TREATMENT					
WHO Western Pacific Region					
Viet Nam	2003–2003	1	0.0	0.0	0.0
ARTEMETHER–LUMEFANTRINE					
WHO African Region					
Angola	2004–2004	2	1.2	0.0	2.3
Benin	2005–2007	4	0.8	0.0	6.5
Burkina Faso	2005–2007	4	4.3	1.9	12.3
Cameroon	2006–2009	6	0.7	0.0	2.3
Comoros	2004–2007	9	0.0	0.0	3.2
Congo	2004–2006	2	1.6	0.0	3.2
Côte d'Ivoire	2005–2009	4	2.1	0.0	7.4
Democratic Republic of the Congo	2005–2008	6	2.4	0.0	9.2
Eritrea	2007–2007	3	0.0	0.0	0.0
Ethiopia	2003–2009	9	0.0	0.0	7.5
Gambia	2002–2008	2	2.0	0.0	3.9
Ghana	2003–2007	5	4.0	1.7	13.8
Kenya	2002–2008	12	2.7	0.0	6.6
Liberia	2007–2007	2	0.0	0.0	0.0
Madagascar	2006–2006	1	1.7	1.7	1.7
Malawi	2005–2005	1	7.1	7.1	7.1
Mali	2004–2008	6	3.0	0.0	6.0

	STUDY YEARS	NUMBER OF STUDIES	MEDIAN	MINIMUM	MAXIMUM
Mozambique	2005–2008	4	1.6	0.0	3.1
Niger	2006–2006	1	4.4	4.4	4.4
Nigeria	2002–2007	5	0.0	0.0	2.0
Rwanda	2004–2007	3	1.5	0.0	6.9
Senegal	2002–2008	6	0.9	0.0	3.2
South Africa	2002–2007	3	0.0	0.0	5.2
Togo	2005–2008	6	1.5	0.0	4.4
Uganda	2002–2008	8	2.3	0.0	8.9
United Republic of Tanzania					
Mainland	2002–2008	8	2.9	0.0	8.6
Zanzibar	2002–2006	3	0.0	0.0	2.7
Zambia	2004–2006	12	0.0	0.0	6.7
Zimbabwe	2007–2007	3	0.0	0.0	1.9
WHO Region of the Americas					
Brazil	2005–2007	2	0.0	0.0	0.0
Ecuador	2005–2006	1	0.0	0.0	0.0
Guyana	2004–2008	2	1.6	0.0	3.2
Suriname	2003–2006	3	2.0	1.9	4.7
Venezuela (Bolivarian Republic of)	2004–2005	1	0.0	0.0	0.0
WHO South-East Asia Region					
Bangladesh [42 d]	2003–2007	3	2.9	0.0	5.7
India	2007–2008	3	0.0	0.0	1.4
Indonesia	2004–2008	3	3.1	0.0	7.7
Myanmar	2004–2009	15	2.5	0.0	8.3
Nepal	2005–2008	2	0.0	0.0	0.0
Thailand	2001–2006	3	0.0	0.0	0.9
WHO Eastern Mediterranean Region					
Sudan					
High-transmission area	2004–2004	1	2.8	2.8	2.8
Low-to-moderate-transmission area	2004–2008	8	0.0	0.0	8.9
Yemen	2007–2007	1	0.0	0.0	0.0
WHO Western Pacific Region					
Cambodia	2001–2004	4	21.3	13.5	28.9
China	2004–2005	1	0.0	0.0	0.0
Lao People's Democratic Republic	2002–2006	4	1.6	0.0	6.3
Papua New Guinea	2006–2007	1	2.7	2.7	2.7
Philippines	2003–2009	9	0.0	0.0	5.6
Solomon Islands	2008–2008	1	0.0	0.0	0.0
Viet Nam	2001–2001	1	2.2	2.2	2.2

	STUDY YEARS	NUMBER OF STUDIES	MEDIAN	MINIMUM	MAXIMUM
ARTEMISININ–PIPERAQUINE					
WHO South-East Asia Region					
Myanmar	2008–2008	1	6.0	6.0	6.0
Thailand	2005–2007	2	1.7	1.6	1.8
ARTESUNATE 5-DAY TREATMENT					
WHO African Region					
Gabon	2002–2004	1	9.5	9.5	9.5
Mali	2002–2004	1	3.5	3.5	3.5
WHO Western Pacific Region					
China	2002–2002	1	20.6	20.6	20.6
Viet Nam	2000–2005	3	12.5	11.9	17.5
ARTESUNATE 7-DAY TREATMENT					
WHO African Region					
Central African Republic	2004–2004	1	5.6	5.6	5.6
WHO Western Pacific Region					
Cambodia	2006–2009	3	6.7	5.2	8.3
China	2009–2009	1	0.0	0.0	0.0
Viet Nam	2002–2009	11	3.2	0.0	14.6
ARTESUNATE–AMODIAQUINE					
WHO African Region					
Angola	2003–2004	3	1.2	0.0	3.3
Benin	2007–2007	1	0.0	0.0	0.0
Burkina Faso	2004–2007	5	4.9	3.9	21.5
Burundi	2005–2006	2	5.2	2.9	7.5
Cameroon	2005–2009	9	3.7	0.0	8.7
Congo	2004–2004	1	1.5	1.5	1.5
Côte d'Ivoire	2008–2009	2	0.0	0.0	0.0
Democratic Republic of the Congo	2003–2005	8	6.2	0.0	19.0
Equatorial Guinea	2006–2006	1	3.3	3.3	3.3
Eritrea	2006–2009	8	4.1	1.5	12.5
Gabon	2004–2005	1	13.8	13.8	13.8
Ghana	2003–2006	4	4.3	0.0	14.0
Guinea	2004–2004	1	1.0	1.0	1.0
Kenya	2004–2007	3	12.3	9.1	13.3
Liberia	2007–2007	2	0.0	0.0	0.0
Madagascar	2006–2007	10	0.0	0.0	8.7
Malawi	2005–2005	2	1.8	0.0	3.6
Mali	2002–2006	4	2.0	0.0	7.6
Nigeria	2004–2006	5	0.0	0.0	7.8

	STUDY YEARS	NUMBER OF STUDIES	MEDIAN	MINIMUM	MAXIMUM
Rwanda	2002–2004	6	6.2	4.7	22.5
Senegal	2002–2008	7	0.0	0.0	0.5
Sierra Leone	2004–2004	1	27.0	27.0	27.0
Togo	2005–2008	6	0.0	0.0	6.1
Uganda	2002–2008	9	5.2	3.8	12.0
United Republic of Tanzania					
Mainland	2004–2007	2	4.4	2.0	6.8
Zanzibar [42 d]	2002–2005	2	12.1	10.8	13.4
WHO Region of the Americas					
Colombia	2000–2006	2	0.0	0.0	0.0
WHO South-East Asia Region					
Indonesia	2003–2006	8	8.8	0.0	24.1
Myanmar	2004–2007	13	3.4	0.0	19.2
WHO Eastern Mediterranean Region					
Afghanistan [42 d]	2002–2003	1	28.4	28.4	28.4
Somalia	2006–2006	2	2.1	1.9	2.2
Sudan					
High-transmission area	2003–2004	2	3.1	1.0	5.1
Low-to-moderate-transmission area	2003–2005	2	6.1	5.0	7.2
Yemen	2004–2004	1	18.5	18.5	18.5
WHO Western Pacific Region					
Viet Nam	2006–2007	1	2.1	2.1	2.1
ARTESUNATE–DOXYCYCLINE					
WHO South-East Asia Region					
Bhutan	2000–2005	11	5.0	0.0	22.4
ARTESUNATE–MEFLOQUINE					
WHO African Region					
Burkina Faso	2007–2008	1	0.0	0.0	0.0
Cameroon	2006–2009	2	1.8	0.0	3.5
Gabon	2005–2006	2	0.0	0.0	0.0
Nigeria	2007–2008	1	2.9	2.9	2.9
Senegal	2008–2008	1	0.0	0.0	0.0
WHO Region of the Americas					
Brazil	2005–2007	3	0.0	0.0	0.0
Colombia	2007–2008	1	0.0	0.0	0.0
Guyana	2004–2005	1	1.2	1.2	1.2
Peru	2003–2006	3	0.0	0.0	0.0
Suriname [35 d]	2002–2003	2	4.1	2.4	5.8
Venezuela (Bolivarian Republic of)	2004–2005	1	0.0	0.0	0.0

	STUDY YEARS	NUMBER OF STUDIES	MEDIAN	MINIMUM	MAXIMUM
WHO South-East Asia Region					
Bangladesh	2003–2003	1	0.9	0.9	0.9
Myanmar	2000–2006	9	1.8	0.0	8.9
Thailand	2001–2009	20	0.5	0.0	10.4
WHO Western Pacific Region					
Cambodia	2001–2010	26	2.4	0.0	14.3
Lao People's Democratic Republic	2002–2004	3	0.0	0.0	0.0
Viet Nam	2001–2008	3	0.0	0.0	1.3
ARTESUNATE–PYRONARIDINE					
WHO African Region					
Democratic Republic of the Congo	2007–2008	1	0.5	0.5	0.5
Gabon	2006–2006	1	0.0	0.0	0.0
Gambia	2007–2008	1	0.0	0.0	0.0
Kenya	2007–2008	1	0.0	0.0	0.0
Mali	2007–2008	1	0.0	0.0	0.0
Mozambique	2007–2008	1	0.0	0.0	0.0
Senegal	2007–2008	1	0.0	0.0	0.0
WHO Western Pacific Region					
Philippines	2007–2008	1	0.0	0.0	0.0
ARTESUNATE–SULFADOXINE–PYRIMETHAMINE					
WHO African Region					
Angola	2003–2003	1	1.2	1.2	1.2
Benin	2003–2005	1	5.6	5.6	5.6
Cameroon	2005–2007	2	3.6	1.4	5.7
Congo	2004–2004	1	9.9	9.9	9.9
Democratic Republic of the Congo	2003–2004	3	19.7	0.0	32.8
Ghana	2002–2002	1	6.1	6.1	6.1
Guinea	2004–2004	1	1.0	1.0	1.0
Kenya	2003–2004	1	15.0	15.0	15.0
Malawi	2003–2005	1	26.0	26.0	26.0
Mali	2002–2006	2	1.6	0.0	3.2
Mozambique [42 d]	2003–2005	1	2.3	2.3	2.3
Rwanda	2001–2006	4	13.1	8.5	33.3
South Africa [42 d]	2004–2004	1	1.1	1.1	1.1
United Republic of Tanzania					
Mainland	2006–2006	2	5.6	4.7	6.4
Zambia	2002–2004	3	17.5	3.5	26.5

	STUDY YEARS	NUMBER OF STUDIES	MEDIAN	MINIMUM	MAXIMUM
WHO Region of the Americas					
Colombia	2002–2006	3	2.1	2.0	3.4
Ecuador	2003–2004	2	0.0	0.0	0.0
Peru	2000–2000	1	1.1	1.1	1.1
WHO South-East Asia Region					
India	2005–2007	9	0.0	0.0	4.0
Sri Lanka	2000–2000	1	0.0	0.0	0.0
WHO European Region					
Tajikistan	2004–2004	1	0.0	0.0	0.0
WHO Eastern Mediterranean Region					
Afghanistan	2004–2006	5	0.0	0.0	0.0
Iran (Islamic Republic of)	2005–2007	4	0.0	0.0	0.0
Pakistan	2004–2008	6	0.0	0.0	3.2
Somalia	2004–2006	3	1.0	0.0	5.1
Sudan					
High-transmission area	2003–2004	2	0.5	0.0	0.9
Low-to-moderate-transmission area	2003–2008	12	1.5	0.0	9.7
Yemen	2007–2007	2	0.8	0.0	1.5
WHO Western Pacific Region					
Papua New Guinea	2006–2007	1	10.0	10.0	10.0
ARTESUNATE–SULFALENE–PYRIMETHAMINE					
WHO African Region					
Burkina Faso	2004–2004	1	2.8	2.8	2.8
Cameroon	2006–2007	1	1.2	1.2	1.2
Côte d'Ivoire	2005–2005	2	0.0	0.0	0.0
Mali	2003–2007	2	0.0	0.0	0.0
Rwanda	2005–2007	2	2.5	1.1	3.9
WHO Eastern Mediterranean Region					
Sudan					
Low-to-moderate-transmission area	2005–2006	2	1.5	0.0	2.9
ATOVAQUONE–PROGUANIL					
WHO African Region					
Ethiopia	2006–2006	1	6.7	6.7	6.7
WHO South-East Asia Region					
Thailand	2004–2005	1	2.2	2.2	2.2
WHO Western Pacific Region					
Cambodia	2008–2009	1	0.0	0.0	0.0
Viet Nam	2001–2002	1	5.2	5.2	5.2

	STUDY YEARS	NUMBER OF STUDIES	MEDIAN	MINIMUM	MAXIMUM
CHLOROQUINE					
WHO African Region					
Angola	2002–2002	1	85.7	85.7	85.7
Benin	2002–2005	6	35.5	15.0	73.9
Chad	2002–2003	2	28.3	23.7	32.8
Congo	2003–2003	1	95.1	95.1	95.1
Gambia	2001–2001	1	30.2	30.2	30.2
Ghana	2003–2003	1	75.0	75.0	75.0
Guinea-Bissau	2001–2004	1	20.0	20.0	20.0
Madagascar	2006–2006	6	51.4	22.4	71.0
Malawi	2005–2005	1	1.3	1.3	1.3
Mali	2002–2004	3	36.8	24.5	90.4
Namibia	2003–2003	3	67.7	55.5	78.7
Niger	2005–2005	1	36.8	36.8	36.8
Nigeria	2002–2005	2	43.3	37.9	48.6
Sierra Leone	2002–2003	3	83.3	58.6	91.1
WHO Region of the Americas					
Honduras	2008–2009	1	0.0	0.0	0.0
Nicaragua	2005–2006	1	0.0	0.0	0.0
WHO South-East Asia Region					
Bangladesh	2004–2004	1	57.7	57.7	57.7
India	2001–2008	85	37.5	0.0	100.0
Indonesia	2001–2002	3	72.5	69.1	81.1
Sri Lanka	2000–2004	4	40.8	10.0	100.0
Timor-Leste	2000–2000	1	63.7	63.7	63.7
WHO European Region					
Tajikistan	2002–2002	1	56.0	56.0	56.0
WHO Eastern Mediterranean Region					
Afghanistan	2002–2002	1	89.5	89.5	89.5
Iran (Islamic Republic of)	2000–2004	4	73.6	61.0	78.3
Pakistan	2000–2005	4	38.4	20.0	82.9
Sudan					
High-transmission area	2001–2002	2	100.0	100.0	100.0
Low-to-moderate-transmission area	2003–2003	1	51.5	51.5	51.5
WHO Western Pacific Region					
China	2005–2006	1	40.6	40.6	40.6
Lao People's Democratic Republic	2001–2001	1	31.3	31.3	31.3
Malaysia	2003–2004	2	61.8	45.2	78.4
Viet Nam	2000–2007	8	19.8	0.0	71.9

	STUDY YEARS	NUMBER OF STUDIES	MEDIAN	MINIMUM	MAXIMUM
CHLOROQUINE–SULFADOXINE–PYRIMETHAMINE					
WHO African Region					
Eritrea	2006–2006	1	50.0	50.0	50.0
Gambia	2001–2002	2	6.4	3.9	8.9
Malawi	2003–2005	1	14.0	14.0	14.0
Nigeria	2004–2004	1	10.0	10.0	10.0
Uganda	2001–2004	11	41.0	22.0	73.0
WHO Region of the Americas					
Ecuador	2003–2003	1	0.0	0.0	0.0
WHO South-East Asia Region					
Bangladesh	2002–2003	6	28.3	12.9	33.0
Indonesia	2001–2004	2	6.2	6.2	6.2
WHO European Region					
Tajikistan	2003–2003	1	2.1	2.1	2.1
WHO Eastern Mediterranean Region					
Afghanistan	2004–2004	2	0.0	0.0	0.0
Iran (Islamic Republic of)	2005–2007	5	1.9	0.0	9.1
Sudan					
Low-to-moderate-transmission area	2003–2003	4	10.2	2.5	36.6
WHO Western Pacific Region					
Lao People's Democratic Republic	2002–2003	1	6.9	6.9	6.9
Malaysia	2000–2004	4	41.4	25.0	62.5
Papua New Guinea	2004–2007	4	16.8	13.3	22.2
Philippines	2001–2007	9	9.3	0.0	20.5
Solomon Islands	2007–2007	2	17.9	11.6	24.2
Vanuatu	2001–2005	5	8.5	0.0	16.7
DIHYDROARTEMISININ 5-DAY TREATMENT					
WHO African Region					
Mali	2001–2003	1	7.9	7.9	7.9
WHO South-East Asia Region					
Myanmar	2003–2003	1	10.5	10.5	10.5
WHO Western Pacific Region					
Viet Nam	2002–2003	1	26.7	26.7	26.7
DIHYDROARTEMISININ 7-DAY TREATMENT					
WHO South-East Asia Region					
Myanmar	2003–2003	1	13.1	13.1	13.1
WHO Western Pacific Region					
China	2001–2002	1	2.9	2.9	2.9

	STUDY YEARS	NUMBER OF STUDIES	MEDIAN	MINIMUM	MAXIMUM
DIHYDROARTEMISININ–PIPERAQUINE					
WHO African Region					
Burkina Faso	2005–2006	2	1.9	1.5	2.2
Cameroon	2007–2007	1	2.3	2.3	2.3
Kenya	2005–2007	2	4.2	0.0	8.3
Mozambique	2005–2006	1	6.9	6.9	6.9
Rwanda	2003–2004	3	1.4	1.2	12.5
Uganda	2005–2008	4	1.4	0.3	5.5
Zambia	2005–2006	1	4.7	4.7	4.7
WHO Region of the Americas					
Peru [63 d]	2003–2005	1	1.8	1.8	1.8
WHO South-East Asia Region					
Indonesia [42 d]	2004–2008	3	4.1	2.7	4.8
Myanmar	2003–2009	4	2.9	0.0	5.0
Thailand [63 d]	2002–2004	4	1.3	0.0	3.9
WHO Western Pacific Region					
Cambodia	2008–2010	5	1.3	0.0	7.9
China	2004–2009	2	0.0	0.0	0.0
Lao People's Democratic Republic	2004–2004	1	0.0	0.0	0.0
Papua New Guinea	2006–2007	1	9.9	9.9	9.9
Viet Nam	2001–2010	14	0.0	0.0	6.1
MEFLOQUINE					
WHO African Region					
Benin	2005–2005	1	2.6	2.6	2.6
Nigeria	2007–2008	1	8.8	8.8	8.8
WHO Region of the Americas					
Brazil	2000–2000	1	2.4	2.4	2.4
Guyana	2004–2005	1	3.6	3.6	3.6
Suriname [35 d]	2002–2002	1	7.3	7.3	7.3
QUININE					
WHO African Region					
Ethiopia	2006–2006	1	10.0	10.0	10.0
WHO Region of the Americas					
Venezuela (Bolivarian Republic of)	2002–2003	3	20.0	9.6	22.2
WHO European Region					
Tajikistan	2003–2003	1	0.0	0.0	0.0
WHO Eastern Mediterranean Region					
Sudan					
Low-to-moderate-transmission area	2002–2003	2	6.5	6.3	6.7

	STUDY YEARS	NUMBER OF STUDIES	MEDIAN	MINIMUM	MAXIMUM
QUININE–DOXYCYCLINE					
WHO Eastern Mediterranean Region					
Pakistan	2003–2004	1	0.0	0.0	0.0
QUININE–TETRACYCLINE					
WHO Western Pacific Region					
Cambodia	2006–2007	1	0.0	0.0	0.0
SULFADOXINE–PYRIMETHAMINE					
WHO African Region					
Angola	2002–2003	2	33.0	27.1	38.8
Benin	2002–2007	8	35.7	3.3	71.7
Botswana	2006–2006	3	24.6	12.2	30.1
Burkina Faso	2003–2004	6	6.1	0.0	12.0
Cameroon	2004–2006	3	32.5	29.9	37.5
Chad	2002–2003	2	10.3	4.3	16.3
Congo	2003–2004	1	31.2	31.2	31.2
Democratic Republic of the Congo	2003–2004	2	33.2	23.0	43.4
Ethiopia	2003–2003	11	71.1	52.8	85.7
Gabon	2000–2006	3	13.5	6.1	14.0
Gambia	2001–2001	1	6.1	6.1	6.1
Ghana	2002–2002	1	28.7	28.7	28.7
Kenya	2002–2004	2	31.7	8.0	55.3
Liberia	2000–2000	1	69.7	69.7	69.7
Madagascar	2003–2007	9	2.7	0.0	12.9
Malawi	2003–2005	3	74.0	68.2	81.7
Mali	2002–2004	3	3.4	2.0	7.0
Mozambique [42 d]	2002–2005	3	25.0	11.6	27.0
Namibia	2003–2003	3	33.4	9.1	36.8
Niger	2005–2006	2	16.7	16.6	16.7
Nigeria	2003–2004	3	25.0	20.6	27.0
Sierra Leone	2002–2003	3	27.7	24.6	48.8
South Africa [42 d]	2000–2002	3	10.0	6.4	88.3
Uganda	2001–2002	2	47.3	37.0	57.6
United Republic of Tanzania					
Mainland	2003–2006	7	43.3	25.5	82.2
Zambia	2004–2005	3	33.9	23.2	34.4
WHO Region of the Americas					
Colombia	2001–2005	2	10.7	5.3	16.1
Ecuador	2001–2003	4	2.0	0.0	17.1
Nicaragua	2005–2006	1	0.0	0.0	0.0
Peru	2000–2002	2	9.5	3.3	15.6

	STUDY YEARS	NUMBER OF STUDIES	MEDIAN	MINIMUM	MAXIMUM
WHO South-East Asia Region					
India	2001–2007	22	13.6	0.0	56.7
Indonesia	2001–2005	6	10.0	4.7	59.1
Nepal	2003–2005	3	19.3	12.1	22.0
Sri Lanka	2002–2002	1	0.0	0.0	0.0
Timor-Leste	2001–2001	1	7.9	7.9	7.9
WHO European Region					
Tajikistan	2002–2002	1	16.0	16.0	16.0
WHO Eastern Mediterranean Region					
Afghanistan	2002–2003	3	8.7	4.0	22.7
Iran (Islamic Republic of)	2001–2001	1	0.0	0.0	0.0
Pakistan	2004–2005	1	56.1	56.1	56.1
Sudan					
High-transmission area	2001–2002	2	43.6	17.2	69.9
Low-to-moderate-transmission area	2001–2003	7	7.6	2.0	31.7
Yemen	2004–2005	3	0.0	0.0	5.0
WHO Western Pacific Region					
Lao People's Democratic Republic	2000–2003	3	24.0	18.7	33.0
Malaysia	2003–2004	2	19.4	16.6	22.2
Philippines	2000–2001	5	42.6	8.5	60.6
Viet Nam	2002–2003	2	26.8	14.9	38.7

Annex 2. Subregions

For the purpose of graphical representation, all malaria-endemic countries were grouped according to geographical proximity. In the scatter plots and the box plots, African countries were grouped into four subregions: western Africa, central Africa, eastern Africa and southern Africa (see below). All other malaria-endemic countries were grouped into one of the following subregions: South America, Central America and the Caribbean, Middle East and Central Asia, South Asia, Greater Mekong, or Island nations of Asia and the Pacific. In all the graphs, the groups are referred to as 'subregions'. In Tables A1.2 and A1.3 in Annex 1, countries are grouped according to WHO region.

AFRICA

Western Africa
Benin, Burkina Faso, Côte d'Ivoire, Gambia, Ghana, Guinea, Guinea-Bissau, Liberia, Mali, Niger, Nigeria, Senegal, Sierra Leone, Togo

Central Africa
Cameroon, Central African Republic, Chad, Congo, Democratic Republic of the Congo, Equatorial Guinea, Gabon

Eastern Africa
Burundi, Eritrea, Ethiopia, Kenya, Rwanda, Somalia, Sudan, Uganda, United Republic of Tanzania

Southern Africa
Angola, Botswana, Comoros, Madagascar, Malawi, Mozambique, Namibia, South Africa, Zambia, Zimbabwe

THE AMERICAS

South America
Brazil, Colombia, Ecuador, Guyana, Peru, Suriname, Venezuela (Bolivarian Republic of)

Central America and the Caribbean
Honduras, Nicaragua

ASIA

Middle East and Central Asia
Afghanistan, Iran (Islamic Republic of), Pakistan, Tajikistan, Yemen

South Asia
Bangladesh, Bhutan, India, Nepal, Sri Lanka

Greater Mekong
Cambodia, China, Lao People's Democratic Republic, Myanmar, Thailand, Viet Nam

Island nations of Asia and the Pacific
Indonesia, Malaysia, Papua New Guinea, Philippines, Solomon Islands, Timor-Leste, Vanuatu

This report provides a comprehensive, global overview of antimalarial drug efficacy and the resistance of malaria parasites to the antimalarial medicines used between 2000 and June 2010. Policy-makers in national ministries of health will benefit from this document, as it provides both a global and a regional picture of the efficacy of the antimalarial medicines currently used in national treatment programmes. In addition, the report will be a reference for scientists, enhancing their understanding of the complexity of antimalarial drug resistance.

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