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Responding to the Challenge of Antimalarial Drug Resistance by Routine Monitoring to Update National Malaria Treatment Policies

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Abstract. Reduced sensitivity of *Plasmodium falciparum* to formerly recommended cheap and well-known antimalarial drugs places an increasing burden on malaria control programs and national health systems in endemic countries. The high costs of the new artemisinin-based combination treatments underline the use of rational and updated malaria treatment policies, but defining and updating such policies requires a sufficient volume of high-quality drug-resistance data collected at national and regional levels. Three main tools are used for drug resistance monitoring, including therapeutic efficacy tests, in vitro tests, and analyses of molecular markers. Data obtained with the therapeutic efficacy test conducted according to the standard protocol of the World Health Organization are most useful for updating national treatment policies, while the in vitro test and molecular markers can provide important additional information about changing patterns of resistance. However, some of the tests are technically demanding, and thus there is a need for more resources for training and capacity building in endemic countries to be able to adequately respond to the challenge of drug resistance.

INTRODUCTION

Global efforts toward controlling malaria are greatly challenged by the increasing spread of antimalarial drug resistance. Use of ineffective antimalarials is thus considered partly responsible for the difficulties in reducing malaria morbidity and mortality. This is a problem particularly in sub-Saharan Africa, where susceptibility of *Plasmodium falciparum* to previously used cheap and commonplace antimalarials, such as chloroquine and sulfadoxine–pyrimethamine (SP), has been declining rapidly in many high-transmission areas.[1] To ensure that malaria control strategies and malaria treatment policies rely on the deployment of effective antimalarials, there is a need for systematic monitoring of antimalarial drug efficacy and drug resistance. Artemisinin-based combination treatments (ACTs) are today the only consistently effective antimalarials available, and as ACTs now become more and more widely used, careful monitoring of their therapeutic efficacy and of any emerging resistance is needed. Preventing development of artemisinin resistance requires that the short-acting artemisinin component is "protected" by a longer-acting partner drug of known high efficacy, and therefore it is important also to keep an eye on the efficacy of the different partner drugs currently recommended for use in artemisinin-based combinations.[2] The aims of this overview are to briefly describe the current situation of drug-resistant malaria and to discuss the available tools for monitoring of drug efficacy and early detection of resistance, emphasizing the importance of a standardized and coordinated use of these tools to update national malaria treatment policies.

MALARIA TREATMENT FAILURE AND DRUG RESISTANCE

Before the spread and assessment of antimalarial drug resistance can be described, a few words on the definition of "resistance" are needed. Resistance of antimalarial drugs is defined as the "the ability of a parasite strain to survive and/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."[3] This definition was originally drawn up before techniques allowing for in vitro drug-sensitivity testing and detection of molecular markers of resistance were available, and thus the definition is based on clinical observation. Today, this is still the case, although confirmation of true resistance to an antimalarial requires proof that the parasites are recrudescent (rather than arising from re-infection) in a patient who recently received treatment and a demonstration that an effective blood concentration of the drug or its metabolites has been maintained for at least 4 parasite cycles.[4] The results of in vitro drug-sensitivity tests and the presence of gene mutations implicated in resistance are additional indicators of resistance (see later), but as these tests are only rarely conducted simultaneously with therapeutic efficacy tests, especially pharmacokinetic drug profiles, merely the failure to clear malarial parasitemia and/or resolve clinical symptoms is conventionally considered to be an indicator of drug resistance. In the event of a failing treatment, it is import to realize, however, that treatment failure may not necessarily be the same as true antimalarial drug resistance. Failure to clear an episode of malaria despite administration of an antimalarial may just be explained by insufficient blood concentrations of the drug, and thus in daily clinical practice, many factors may explain a treatment failure, such as incorrect dosing, problems of patient compliance, poor drug quality, interactions with other drugs, inter-individual variation in pharmacokinetics including poor absorption, rapid elimination (due to diarrhea or vomiting), or insufficient biotransformation of prodrugs because of human genetic characteristics. Furthermore, it should be considered whether a "failure" of malarial treatment may only be a matter of misdiagnosis of the patient.

SUMMARY OF THE EPIDEMIOLOGY OF DRUG-RESISTANT MALARIA

During the last 50 years, resistance to antimalarial drugs has been documented for *P. falciparum, P. vivax*, and recently

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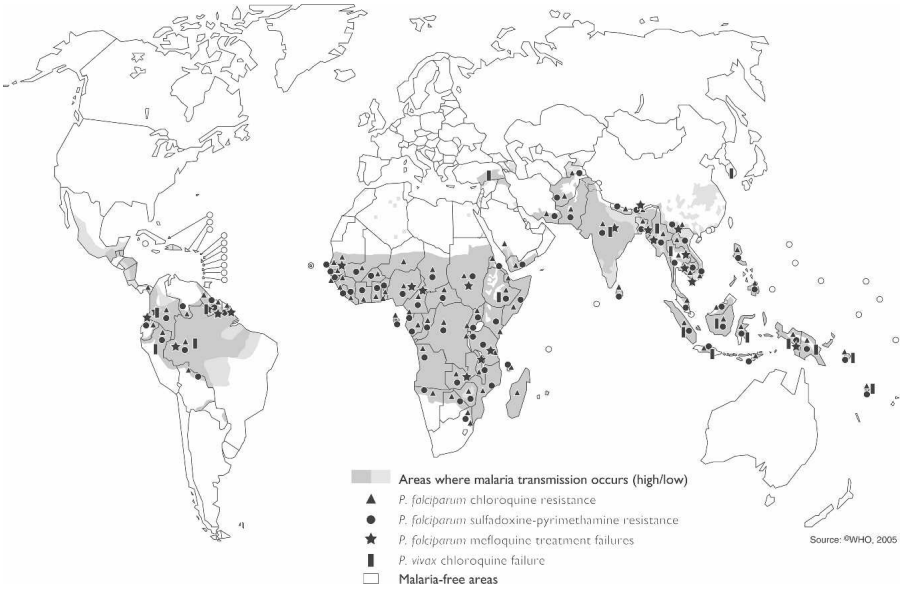

FIGURE 1. Malaria transmission and reported treatment failure and drug resistance in 2005. (The designations employed and the presentation of material on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city, or area or of its authorities, or concerning delimitation of its frontiers or boundaries. Dashed lines represent approximate border lines for which there may not yet be full agreement.)

also *P. malariae* (Figure 1). Resistance of *P. falciparum* has been observed to almost all currently used antimalarials except for artemisinin and its derivatives, although the geographical distributions and rates of spread have varied considerably between regions and countries. The spread of resistance to some of the common antimalarials is briefly summarized in the following, and a detailed account of the global levels of resistance can be found in a recent WHO report.[5]

***P. falciparum* resistance.** Chloroquine resistance has been reported from all falciparum-endemic areas with the exception of Central America and the Caribbean. Resistance was first documented on the border between Thailand and Cambodia and in Columbia in the late 1950s and early 1960s. Since then, chloroquine resistance has spread throughout the tropical world. In Africa, chloroquine resistance was first detected in Tanzania in the late 1970s, and has since spread and intensified across the continent. Today, median clinical failure rates of chloroquine as high as 70–80% are found in several countries in both Asia, Africa, and the Americas. Amodiaquine is generally more effective than chloroquine. However, there is cross-resistance between the two drugs, and the efficacy of amodiaquine is declining in many areas. Very high levels of resistance to SP are found in many parts of southeast Asia, eastern and southern Africa, and the Amazon region. Despite widespread use of quinine, resistance levels are low, and quinine is still generally effective. Mefloquine resistance is prevalent in Myanmar, Thailand, Cambodia, and Vietnam. In the Amazon region, low-level mefloquine resistance has been reported. In Africa, mefloquine resistance is rare, but a few prophylactic and treatment failures have been observed. Confirmed resistance to artemisinin and its derivatives (artemether, artesunate, and dihydroartemisinin) has not been reported.

***P. vivax* and *P. malariae* resistance.** Resistance of *P. vivax* is rare and generally limited to chloroquine, which was first reported in the late 1980s in Papua New Guinea and Indonesia. Focal true chloroquine resistance (with whole-blood chloroquine + desethylchloroquine concentrations > 100 ng/mL at day of failure) or prophylactic and/or treatment failure not necessarily in relation with true resistance have later also been observed in other parts of the world.[6] Resistance of *P. malaria*e to chloroquine was observed recently in Indonesia.[7] *P. vivax* has shown innate resistance to sulfadoxine but developed additional resistance to sulfadoxine and pyrimethamine in many areas.[8,9]

TOOLS FOR MONITORING

The increasing spread of antimalarial drug resistance has emphasized the need for systematic monitoring to suggest where malaria treatment policies should be revised to secure rational use and effective case management. The available monitoring procedures include first of all the therapeutic efficacy test (also known as the in vivo test), which involves the repeated assessment of clinical and parasitological outcomes of treatment during a fixed period of follow-up to detect any reappearance of symptoms and signs of clinical malaria and/or parasites in the blood. Other available methods include in vitro studies of parasite susceptibility to drugs in culture and studies with molecular methods of gene mutations or gene amplifications associated with parasite resistance (Table 1).

Therapeutic efficacy testing. *Therapeutic efficacy testing in* P. falciparum. The therapeutic efficacy test remains the gold standard for determining antimalarial drug efficacy for the management of *P. falciparum* infections. It provides policy makers and national malaria control programs a straightforward indicator of the efficacy of an antimalarial drug or a combination treatment in a given population at risk, to suggest whether a drug is still appropriate as first- or second-line treatment. Studies of therapeutic efficacy are relatively straightforward to set up, although they tend to be lengthy

TABLE 1
Tools for monitoring antimalarial drug efficacy and drug resistance

	Therapeutic efficacy test	In vitro sensitivity assay	Molecular markers
Definition	Treatment of symptomatic *P. falciparum*-infected patients 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 gene mutation(s) or amplification that modify drug-target (enzymes) or drug-tranporter functions or affinities (genetic characterization of drug targets or transport)
Indications	Gold standard for monitoring antimalarial drug efficacy and for guiding drug policy	Detect reduced parasite response to antimalarial drug Early warning system (adjunct to therapeutic efficacy test)	Detect resistance-related mutations or amplification Early warning system (adjunct to therapeutic efficacy test)
Advantages	Easily interpretable results Simple method with minimal training required (except microscopy) Minimal equipment and supplies required Relatively inexpensive to conduct (depending on local conditions) if integrated into the national malaria control programmes	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 (e.g., atovaquone) Mutations precedes in vivo resistance
Drawbacks	Interference of immunity, previous drug intake, variation of drug absorption or metabolism Misclassification of reinfection and recrudescence Treatment failures do not 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 ability to compare between sites Long duration of patient monitoring may result in high patient loss to follow-up	Correlation with therapeutic efficacy test not fully established Presence of mixed population with different drug sensitivity phenotypes Expensive equipment and supplies required Training required Numerous available methods but not always comparable Lack of standardized in vitro protocol Threshold of resistance not validated	Correlation with therapeutic efficacy test 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 PCR protocol, including sample collection and DNA extraction

and may be costly, unless integrated into a national malaria control program; in particular, medical and technical personnel (especially microscopists) must be trained. To interpret the results uniformly to follow trends over time and to compare levels of resistance between different regions, studies must be carried out with the same standardized protocol, at the same sentinel sites, in the same age groups, and, if possible, at the same time of year in a given site.[10,11]

The WHO standard protocol is meant for the evaluation of antimalarial drugs or drug combinations (chloroquine, amodiaquine, quinine, SP, ACTs, etc.) for treatment of uncomplicated *P. falciparum* malaria. The design is simple: a one-armed prospective study of clinical and parasitological responses after administration of antimalarial treatment to children of age 6–59 months with a degree of immunity that is unlikely to have much impact on the outcome of the test. In areas of low or moderate transmission in which it is difficult or time-consuming to enroll enough children in this age group, children > 5 years old and adults can be included, although it should be borne in mind that the results will be biased, as adults always respond better than children. To avoid the inclusion of asymptomatic carriers, only patients with 2000 parasites per μL or more (1000 parasites per μL in areas of low or moderate transmission) are included. Calculation of the sample size required by estimating prevalence is now preferred to the lot quality assurance sampling method. If the selected confidence interval is 95% with a precision of 10%, the sample size ranges between 50 and 100 patients. A minimum of 50 patients must be enrolled in order for the sample to be representative. This sample size was appropriate to detect failure cases during routine monitoring of the efficacy of currently recommended ACTs after their implementation. The recommended duration of follow-up is $\geq$ 28 days in areas of intense as well as low-to-moderate malaria transmission. For treatment with drugs such as amodiaquine, chloroquine, and SP, a 28-day follow-up is considered appropriate; for slowly eliminated antimalarials (lumefantrine, mefloquine, piperaquine, pyronaridine), recrudescences may occur after 28 days, and so 42- or even 63-day follow-up periods are recommended.

Several changes to the WHO protocol have been introduced based on the feedback from the field since 1996.[10,12] The main difference between the protocols for areas of high transmission and for areas of low-to-moderate transmission, apart from the inclusion criteria, was in the management of parasitological failures without clinical signs. The differences reflect regional program priorities (treatment of clinical cure or for radical cure). In 2005, the malaria treatment guidelines stressed that the objective of antimalarial treatment, even in areas of high transmission, should be radical cure of the disease.[2] From a clinical point of view, the persistence of parasites resulting from treatment failure can lead to anemia, gametocyte carriage, and risk of recurrent clinical signs and symptoms. These 3 harmful consequences of treatment failure are taken into consideration in the modified 2005 WHO protocol.

Patients included in the efficacy study and not lost to follow-up or excluded (e.g., because of self-medication, development of concomitant febrile infections, or refusal to continue participation) are classified in one of the following categories: early treatment failure, late clinical failure, late parasitological failure, or adequate clinical and parasitological response. These classifications rely on the presence or absence of fever or other signs of clinical malaria and/or presence of parasitemia during the course of follow-up, as listed in Table 2. The rates of total failure are used to define cut-off points for drug-policy change. Monitoring antimalarial drug efficacy is based on presence or absence of asexual parasites detected by microscopy at admission and during the follow-up, and the use of rapid diagnostic tests has been suggested for the screening of febrile patients. So far, however, rapid tests cannot be used during the follow-up because quantitative parasitemia is needed to classify patient outcome as early treatment failure, and some tests show false-positive results related to the persistence of circulating antigens or presence of gametocytes, despite complete parasite clearance.

TABLE 2

Classification of treatment outcome according to the WHO Protocol 2005 for all endemic areas

Early treatment failure
• Danger signs or severe malaria on day 1, 2, or 3, in the presence of parasitemia;
• Parasitemia on day 2 higher than on day 0, irrespective of axillary temperature;
• Parasitemia on day 3 with axillary temperature $\geq$ 37.5°C;
• Parasitemia on day 3 $\geq$ 25% of count on day 0.
Late clinical failure
• Danger signs or severe malaria in the presence of parasitemia on any day between days 4 and 28 (42 or 63), without the patient previously meeting any of the criteria of early treatment failure;
• Axillary temperature $\geq$ 37.5°C in the presence of parasitemia on any day between days 4 and 28 (42 or 63), without the patient previously meeting any of the criteria of early treatment failure.
Late parasitological failure
• Presence of parasitemia between days 7 and 28 (42 or 63) with temperature < 37.5°C, without the patient previously meeting any of the criteria of early treatment failure or late clinical failure.
Adequate clinical and parasitological response
• Absence of parasitemia on day 28 (42 or 63), irrespective of axillary temperature, without the patient meeting any of the criteria of early treatment failure, late clinical failure, or late parasitological failure.

Therapeutic efficacy testing in P. vivax, P. ovale*, and* P. malariae. Relapse, re-infection, and recrudescence cannot be distinguished reliably in the infections with *P. vivax* and *P. ovale*. Nevertheless, in vivo assessments of chloroquine susceptibility can be performed using the same protocol format as for *P. falciparum*, with a follow-up period of 28 days and preferably accompanied by measurement of whole-blood chloroquine and desethychloroquine levels at the day of failure. Recurrent infections within this period presenting with whole-blood chloroquine + desethychloroquine concentrations exceeding 100 ng/mL are currently considered as resistant whether they are a relapse, a recrudescence, or even a new infection, as this concentration should be suppressive.[13] This definition will most probably need to be reconsidered according to the on-going trials.

WHO database on therapeutic efficacy studies. To facilitate the use of data from such standardized assessments, a comprehensive database has been established by WHO, where reported standardized drug-efficacy results are compiled and summarized to better inform endemic countries and their national malaria control programs about the current situation and of changing patterns of resistance. The data in the database originate from 3 sources: 1) published studies; 2) unpublished studies (available in reports by ministries of health, national control programs, or nongovernmental organizations, consultant reports, theses, and papers, or posters presented at national and international conferences); and 3) regular data from surveillance studies conducted according to the WHO standard protocol and sent from countries to WHO for validation. On the basis of this database, detailed estimates of global levels of drug efficacy in the period from 1996 to 2004 were recently reported as mentioned above.[5]

In vitro tests. To support the evidence of a failing antimalarial, an in vitro test can be used to provide a more accurate measure of drug sensitivity under controlled experimental conditions. Parasites are here exposed to precisely known concentrations of an antimalarial drug and are observed for inhibition of maturation into schizonts. Several in vitro tests exist, and they differ primarily in how their results are interpreted. These include microscopic examination of blood films for the WHO Mark III test, the radioisotopic test, and the enzyme-linked immunosorbent assay with antibodies directed against *Plasmodium* lactate dehydrogenase or histidine-rich protein II and more recently the Sybr Green test.[14]

In vitro tests overcomes some of the many confounding factors influencing the results of in vivo tests, such as subtherapeutic drug concentrations and the influence of host factors on parasite growth (e.g., factors related to acquired immunity), and therefore provide a more accurate picture of the "true" level of resistance to the drug. Multiple tests can be performed on parasite isolates, using several drugs and drug combinations simultaneously. However, in part because in vitro tests do not include host factors, the correlation between results of in vitro and in vivo tests is not consistent and is not well understood. In vitro tests have proven useful as part of epidemiological monitoring, including monitoring of cross-resistance patterns between different antimalarials in a region, monitoring of baseline drug sensitivity before it is introduced as part of national policy, in studies of temporal and spatial changes for early warning and guidance on need for therapeutic efficacy studies, and for the validation of molecu-

lar markers of resistance. It is often difficult to compare results, even from laboratories where the same type of test is used, because the results—which are usually expressed as the 50% inhibitory concentration (IC_{50}), the 90% inhibitory concentration (IC_{90}), or the MIC—are the expression of more than 10 factors that are rarely identical in different laboratories. To date, few thresholds of resistance have been correctly validated. Moreover, a cut-off point validated with a given test is valid only for that assay system, with its specific in vitro factors and cannot be extrapolated to another test. To validate the in vitro cut-off point for resistance, the results of in vitro tests and of therapeutic efficacy tests conducted in a nonimmune population (children or travelers) must be compared in a sufficiently large sample. Furthermore, patient follow-up must be sufficiently long, and every effort must be made to confirm that failures are not due to insufficient drug absorption, re-infection, or other causes unrelated to drug resistance. Therefore, results should not be expressed as percentage resistance, especially when the thresholds of resistance are not validated. Instead, they should be expressed as a geometric mean of the IC_{50} or MIC, which allows a more precise quantitative comparison of sites in a given country and over time.

Molecular markers. *Genetic markers of resistance.* As an additional means of detecting changing pattens of resistance, molecular tests have been developed in recent years for the detection of parasite gene mutations or amplifications associated with resistance to a number of antimalarials. These tests are based on PCR analyses of only small amounts of parasite DNA material in finger-prick blood dried on filter paper, one sample allowing for multiple tests to be performed and molecular targets of several drugs to be characterized. The tests are easier to run than in vitro tests and are thus more readily deployed routinely by malaria control programs, although technical capacity is still required. Information on the prevalence of gene mutations may give an indication of the level of drug resistance in an area as a mean of early warning, and relatively well-defined molecular markers of resistance have been established for pyrimethamine (dihydrofolate reductase, *dhfr*), sulfadoxine (dihydropteroate synthase, *dhps*), and chloroquine (*P. falciparum* chloroquine-resistance transporter, *Pfcrt*).[15] An increased number of *P. falciparum* multidrug-resistance gene 1 (*Pfmdr1*) has been identified as a marker of mefloquine resistance, and mutations in *Pfmdr1* have also been implicated in modification of the sensitivity to amino alcohols and artemisinins, although field studies have provided contradictory results.[16,17] Treatment failures observed with atovaquone–proguanil have been associated with a specific point mutation in the *cytochrome b* gene at codon 268, but other reported treatment failures with this drug were not associated with this mutation.[18,19] Although monitoring of molecular markers of resistance from a programmatic point is simpler than in vitro sensitivity testing, the molecular methods also have their drawbacks. An important problem is that the mutations detected and the measured therapeutic efficacy do not always correlate well, as many factors determine the therapeutic response in addition to parasite sensitivity to the antimalarial treatment. To be useful as a public-health measure, the molecular markers should reliably predict the clinical and parasitological outcome of treatment, but at present the markers do not give an accurate prediction in all epidemiologic settings. However, serial assessment of molecular markers can be a useful guide to the emergence of resistance, especially if used consistently over time in comparable study populations to detect trends. With newly developed high-throughput methods,[20,21] more comprehensive population-based analyses will be possible, which may lead to a better understanding of the different genetic markers of resistance and their use in prediction of drug efficacy.[22]

Genetic markers of recrudescence and re-infection. Detection of genetic parasite markers also provides a valuable tool in support of monitoring programs that rely on therapeutic efficacy testing. When monitoring of therapeutic efficacy is extended beyond 14 days in an intense transmission area or with 28-day follow-up in a low-to-moderate transmission area, and as most antimalarial drugs have no effect on the liver stages of *P. falciparum*, methods are needed to distinguish cases of new infection from recrudescence. PCR methods are relatively simple means of analyzing genetict diversity by analyzing 3 highly polymorphic genes: merozoite surface proteins 1 and 2 (*msp-1* and *msp-2*) and glutamate-rich protein (*glurp*).[23] However, PCR analyses are still limited primarily to research-oriented monitoring projects and have not yet become part of routine efficacy monitoring in many places. Thus, there is a need to build technical and human capacity at national levels to provide this testing service for sentinel sites.

COORDINATED MONITORING AT NATIONAL AND REGIONAL LEVEL

To allow for the collection of accurate and comparable data on drug resistance, WHO recommends a systematic and uniform organization of drug-efficacy monitoring, coordinated by ministries of health and national malaria control programs. Many countries are already collecting drug-efficacy data in a systematic and timely manner, and monitoring systems in other countries can be improved, even if only limited resources are available. National monitoring of drug efficacy mainly requires a well-planned and coordinated system for resistance-data collection, including established sentinel sites with the necessary trained staff, some minimum laboratory facilities, and agreed standards for data collection, analysis, and reporting of results according to the WHO standard efficacy protocol. Monitoring may involve teams working centrally, which then conduct studies across the country, or teams working at the district level with support from teams at the central level. In both cases, the initial and regular training of central teams is crucial to avoid fundamental errors such as sampling errors, incorrect patient follow-up, or incorrect classification of therapeutic responses. Only the WHO standard efficacy testing protocol should be used for monitoring, from which no deviations should be made that would disturb the direct comparison of results between studies and therefore complicate guidance of national treatment policy. Additional research questions, in particular during new antimalarial drug phase III studies, may be addressed as part of the studies, but the collection of additional information to serve this purpose should not affect the collection of recommended routine data, which serves the purpose of national monitoring. For the comparability of results from year to year and from one site to another, similar study populations must be enrolled, and the studies should be conducted during the same malaria trans-

mission season. Only standard drug administration and regimens should be tested, using drugs of normal standard formulation and quality. In all areas of transmission, 28 days of follow-up should also be the standard. The selection of testing sites should be done carefully to cover different geographical areas and different levels of transmission intensity and may also need to consider operational feasibility to allow a high quality of patient follow-up. Although no definitive scientific advice can be given regarding the number of sites needed, experience suggests that a balance between representativeness and practicality can be achieved with 4–8 sites in a country collecting data for the purpose of policy change. For routine monitoring after the implementation of an ACT, a more limited number of sites is necessary. But a new site can be created in case of an epidemic outbreak or when the distribution of confirmed malaria treatment failures is reported to be higher in some areas of the country through simplified routine surveillance or health information systems. Based on accumulated experience rather than definitive science, it is recommended that assessments be conducted at least once every 2 years, unless the data show a trend of increasing failure rate. Some programs conducting sentinel site surveillance prefer to alternate test sites, for example, testing 4 sites per year with each site being assessed every other year. Other programs find it more easy and sustainable to monitor a first-line drug in all sites the first year and the second-line drug the next year. Both first- and second-line drugs should be monitored because the second-line drug is not always an ACT and because most of the current ACTs and their partner drugs as monotherapies are available in the private sector.

To better allow for the detection of changing patterns of resistance at national, subregional, and regional levels, increased collaboration between national institutions has proven useful. The East Africa Network for Monitoring of Antimalarial Treatment (EANMAT)[24] serves as a good example of such coordinated efforts

UPDATING NATIONAL MALARIA TREATMENT POLICIES

The cut-off points for changing a treatment policy, which are sometimes based on parasitological or economic parameters, are often determined arbitrarily. Such suggested cut-off points are indicative, and decision makers at the national level should feel free to initiate change at any time. In the past, many countries were too slow or too cautious in deciding on policy change, despite a high failure rate with chloroquine reported in the field. In 1999, a scale of dynamic change was established on the basis of clinical failure rates on day 14, but it should be noted that this system was drawn up for regions of high transmission.[25] The WHO malaria treatment guidelines from 2005 suggest that, with the introduction of more effective combination therapies, efficacy of an antimalarial treatment should reach 95% and that a policy change should be seriously considered if the efficacy is < 90% on day 28.[2]

Between 2001 and 2006, 68 countries (nearly 40 in Africa) have changed their policies. Although it might be clear from surveillance at sentinel sites that a first-line treatment is ineffective, the choice of a replacement drug or drug combination at consensus meetings has sometimes proved difficult. According to the 2001 WHO recommendations, an endemic country in which drug resistance to monotherapy is observed should change to a combination therapy based on artemisinin. The currently recommended options are artemether–lumefantrine, artesunate + amodiaquine, artesunate + SP, amodiaquine + SP, and artesunate + mefloquine.[26]

Results of tests for therapeutic efficacy are the most important information for determining whether an antimalarial drug is still effective. Surveillance of therapeutic efficacy over time is an essential component of malaria control. With a few exceptions, all policy changes have been based on the results of in vivo tests. In South Africa, data on in vitro drug sensitivity were also taken into consideration when chloroquine was abandoned as a first-line drug. In Mali, the results of use of molecular markers were determinant in deciding to use sulfadoxine–pyrimethamine instead of chloroquine during an epidemic outbreak.[27] In vitro tests and detection of genetic markers of resistance conducted over time can provide early warning of impending resistance before it becomes clinically apparent and can help guide therapeutic efficacy studies.[28] These tests are also useful for monitoring changes over time in susceptibility to a drug that has been withdrawn.[29] The usefulness of these tests has become evident with the ever-increasing use of combination therapy. It is often impossible to conduct therapeutic efficacy tests for each component, owing to ethical problems, nonavailability of the drug as a single therapy, and the need to study a large number of patients. In vitro tests can be used to monitor susceptibility to each drug in a combination.

FUTURE CHALLENGES

The need for high-quality monitoring of antimalarial drug resistance has increased in recent years. It is increasingly important to know if the required high levels of efficacy of the new and costly combination treatments are maintained, and a more proactive approach to malaria treatment policy change is considered a key to effective malaria control. Technical advances in recent years have made it possible to provide more accurate efficacy results than previously, but the technical requirements in routine monitoring place an additional burden on malaria control efforts, such as the increase in the recommended duration of follow-up and the need of PCR analysis, which will inevitably increase the cost of routine monitoring. Sustained funding for routine monitoring activities is becoming a major issue. In the last decade, countries had to carry out a limited numbers of studies to demonstrate that the monotherapies used as first- and second-line drugs were failing and to compile the baseline data on ACT efficacy. Monitoring ACTs is becoming crucial because the long-acting partner drug of some of the recommended ACTs are marketed as monotherapies, and their efficacy is compromised by high resistance rates, exposing the artemisinin derivatives to the development of resistance. Funding agencies should request the countries to include systematically in their proposals a budget for monitoring activities, particularly when ACTs are going to be implemented at a large scale.

National malaria control programs should be collecting information on the cost of testing to better support their requests for funding. Despite support from the Global Fund, the World Bank or the President's Malaria Initiative, many countries are still facing either a funding gap for monitoring

drug efficacy or an absence of sustainability in funding for this specific activity. When constraints caused by limited budgets or limited numbers of qualified personnel are difficult to overcome, the available resources should be channeled into more complete and regular studies at fewer sites to obtain better information.

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