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Drug resistance in malaria

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Response**

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Drug resistance in malaria

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A BACKGROUND DOCUMENT FOR
THE WHO GLOBAL STRATEGY
FOR CONTAINMENT OF
ANTIMICROBIAL
RESISTANCE

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Contents

1. Introduction	1
2. Disease incidence and trends	2
2.1 Geographical distribution and populations at risk	2
2.2 Causative agents	3
2.3 Diagnosis	3
2.3.1 Microscopy	3
2.3.2 Clinical (presumptive) diagnosis	3
2.3.3 Antigen detection tests	5
2.3.4 Molecular tests	5
2.3.5 Serology	5
2.4 Drugs available for treatment of malaria	5
2.4.1 Quinine and related compounds	5
2.4.2 Antifolate combination drugs	9
2.4.3 Antibiotics	9
2.4.4 Artemisinin compounds	9
2.4.5 Miscellaneous compounds	9
2.4.6 Combination therapy with antimalarials	10
2.5 Current status of drug-resistant malaria	10
3. Causes of resistance	12
3.1 Definition of antimalarial drug resistance	12
3.2 Malaria treatment failure	12
3.3 Mechanisms of antimalarial resistance	12
3.3.1 Chloroquine resistance	12
3.3.2 Antifolate combination drugs	13
3.3.3 Atovaquone	13
3.4 Factors contributing to the spread of resistance	13
3.4.1 Biological influences on resistance	13
3.4.2 Programmatic influences on resistance	15
4. Detection of resistance	16
4.1 <i>In vivo</i> tests	16
4.2 <i>In vitro</i> tests	17
4.3 Animal model studies	17
4.4 Molecular techniques	17
4.5 Case reports and passive detection of treatment failure	18
5. Treatment	19
6. The future: prevention of drug resistance	20
6.1 Preventing drug resistance	20
6.1.1 Reducing overall drug pressure	21
6.1.2 Improving the way drugs are used	21
6.1.3 Combination therapy	21

7. Conclusions and recommendations	23
7.1 Priorities	23
8. Bibliography	24
Figure and tables	
Figure 1. Approximate distribution of malaria	2
Table 1. Comparative descriptions of available malaria diagnostic methods	4
Table 2. Antimalarial drugs for uncomplicated malaria	6
Table 3. Distribution of drug-resistant <i>Plasmodium falciparum</i> malaria	10

1. Introduction

Malaria remains an important public health concern in countries where transmission occurs regularly, as well as in areas where transmission has been largely controlled or eliminated. Malaria is a complex disease that varies widely in epidemiology and clinical manifestation in different parts of the world. This variability is the result of factors such as the species of malaria parasites that occur in a given area, their susceptibility to commonly used or available antimalarial drugs, the distribution and efficiency of mosquito vectors, climate and other environmental conditions and the behaviour and level of acquired immunity of the exposed human populations. In particular, young children, pregnant women, and non-immune visitors to malarious areas are at greatest risk of severe or fatal illness. Many malaria control strategies exist, but none are appropriate and affordable in all contexts. Malaria control and prevention efforts need to be designed for the specific environment in which they will be used and need to take into account the local epidemiology of malaria and the level of available resources and political will.

Antimalarial drug resistance has emerged as one

of the greatest challenges facing malaria control today. Drug resistance has been implicated in the spread of malaria to new areas and re-emergence of malaria in areas where the disease had been eradicated. Drug resistance has also played a significant role in the occurrence and severity of epidemics in some parts of the world. Population movement has introduced resistant parasites to areas previously free of drug resistance. The economics of developing new pharmaceuticals for tropical diseases, including malaria, are such that there is a great disparity between the public health importance of the disease and the amount of resources invested in developing new cures (1, 2). This disparity comes at a time when malaria parasites have demonstrated some level of resistance to almost every anti-malarial drug currently available, significantly increasing the cost and complexity of achieving parasitological cure.

The purpose of this review is to describe the state of knowledge regarding drug-resistant malaria and to outline the current thinking regarding strategies to limit the advent, spread, and intensification of drug-resistant malaria.

2. Disease incidence and trends

2.1 Geographical distribution and populations at risk

Malaria occurs in over 90 countries worldwide. According to figures provided by the World Health Organization (3), 36% of the global population live in areas where there is risk of malaria transmission, 7% reside in areas where malaria has never been under meaningful control, and 29% live in areas where malaria was once transmitted at low levels or not at all, but where significant transmission has been re-established (3). The development and spread of drug-resistant strains of malaria parasites has been identified as a key factor in this resurgence and is one of the greatest challenges to malaria control today. Although there is currently an increase in attention and resources aimed at malaria, including such initiatives as Roll Back Malaria (4), the Multilateral Initiative on Malaria

(5) and the Medicines for Malaria Venture (6) a history of unpredictable support for malaria-related research and control activities in endemic countries have left many of these countries with little technical capacity for malaria control activities.

Each year an estimated 300 to 500 million clinical cases of malaria occur, making it one of the most common infectious diseases worldwide. Malaria can be, in certain epidemiological circumstances, a devastating disease with high morbidity and mortality, demanding a rapid, comprehensive response. In other settings, it can be a more pernicious public health threat. In many malarious areas of the world, especially sub-Saharan Africa, malaria is ranked among the most frequent causes of morbidity and mortality among children and is often the leading identifiable cause. WHO estimates that more than 90% of the 1.5 to 2.0 million deaths

FIGURE 1. APPROXIMATE DISTRIBUTION OF MALARIA



attributed to malaria each year occur in African children (3). Other estimates based on a more rigorous attempt to calculate the burden of disease in Africa support this level of mortality (7). In addition to its burden in terms of morbidity and mortality, the economic effects of malaria infection can be tremendous. These include direct costs for treatment and prevention, as well as indirect costs such as lost productivity from morbidity and mortality, time spent seeking treatment, and diversion of household resources. The annual economic burden of malaria infection in 1995 was estimated at US\$.8 billion, for Africa alone (8). This heavy toll can hinder economic and community development activities throughout the region.

Malaria transmission occurs primarily in tropical and subtropical regions in sub-Saharan Africa, Central and South America, the Caribbean island of Hispaniola, the Middle East, the Indian subcontinent, South-East Asia, and Oceania (figure 1). In areas where malaria occurs, however, there is considerable variation in the intensity of transmission and risk of malaria infection. Highland (>1500 m) and arid areas (<1000 mm rainfall/year) typically have less malaria, although they are also prone to epidemic malaria when parasitaemic individuals provide a source of infection and climate conditions are favourable to mosquito development (3). Although urban areas have typically been at lower risk, explosive, unplanned population growth has contributed to the growing problem of urban malaria transmission (9).

2.2 Causative agents

In humans, malaria infection is caused by one or more of four species of intracellular protozoan parasite. *Plasmodium falciparum*, *P. vivax*, *P. ovale*, and *P. malariae* differ in geographical distribution, microscopic appearance, clinical features (periodicity of infection, potential for severe disease, and ability to cause relapses), and potential for development of resistance to antimalarial drugs. To date, drug resistance has only been documented in two of the four species, *P. falciparum* and *P. vivax*.

2.3 Diagnosis (Table 1)

Direct microscopic examination of intracellular parasites on stained blood films is the current standard for definitive diagnosis in nearly all settings. However, several other approaches exist or are in development and will be described here.

2.3.1 Microscopy

Simple light microscopic examination of Giemsa-stained blood films is the most widely practised and useful method for definitive malaria diagnosis. Advantages include differentiation between species, quantification of the parasite density, and ability to distinguish clinically important asexual parasite stages from gametocytes which may persist without causing symptoms. These advantages can be critical for proper case-management and evaluating parasitological response to treatment. Specific disadvantages are that slide collection, staining, and reading can be time-consuming and microscopists need to be trained and supervised to ensure consistent reliability. While availability of microscopic diagnosis has been shown to reduce drug use in some trial settings (10), in practice, results are often disregarded by clinicians (11). Any programme aimed at improving the availability of reliable microscopy should also retrain clinicians in the use and interpretation of microscopic diagnosis.

A second method is a modification of light microscopy called the quantitative buffy coat method (QBC™, Becton-Dickinson). Originally developed to screen large numbers of specimens for complete blood cell counts, this method has been adapted for malaria diagnosis (12). The technique uses microhaematocrit tubes pre-coated with fluorescent acridine orange stain to highlight malaria parasites. With centrifugation, parasites are concentrated at a predictable location. Advantages to QBC are that less training is required to operate the system than for reading Giemsa-stained blood films, and the test is typically quicker to perform than normal light microscopy. Field trials have shown that the QBC system may be marginally more sensitive than conventional microscopy under ideal conditions (13, 14). Disadvantages are that electricity is always required, special equipment and supplies are needed, the per-test cost is higher than simple light microscopy, and species-specific diagnosis is not reliable.

2.3.2 Clinical (presumptive) diagnosis

Although reliable diagnosis cannot be made on the basis of signs and symptoms alone because of the non-specific nature of clinical malaria, clinical diagnosis of malaria is common in many malarious areas. In much of the malaria-endemic world, resources and trained health personnel are so scarce that presumptive clinical diagnosis is the only real-

TABLE 1. COMPARATIVE DESCRIPTIONS OF AVAILABLE MALARIA DIAGNOSTIC METHODS

Method	Sensitivity/specificity	Advantages	Disadvantages	Cost*	References
Rapid diagnostic test based on pLDH: (OptiMal - Flow Inc)	sens: spec:	<ul style="list-style-type: none"> • Differentiates <i>P. falciparum</i> from non-falciparum infections. • Speed and ease of use; minimal training requirements to achieve reliable result. • Reportedly does not remain positive after clearance of parasites. • No electricity, no special equipment needed; could be used in community outreach programmes. 	<ul style="list-style-type: none"> • Cannot differentiate between non-falciparum species. • Will not quantify parasitaemia (+/- only). 	1.00	
Rapid diagnostic stick test based on PfHRP-II: (<i>ParaSight-F</i> - Becton - Dickinson; Malaria PfTest - ICT Diagnostics)	sens: 84% - 97% spec: 81% - 100% lower values probably due to low parasite densities	<ul style="list-style-type: none"> • Speed and ease of use; minimal training requirements to achieve reliable results. • No electricity, no special equipment needed; could be used at health post/ community outreach. • Card format easier to use for individual tests; dipstick test easier to use for batched testing. 	<ul style="list-style-type: none"> • Will not diagnose non-falciparum malaria although subsequent generation tests will be able to do this. • Will not quantify parasitaemia (+/- only). • Can remain positive after clearance of parasites. 	0.80 to 1.00	(23) (22)
Light microscopy	Optimal conditions: sens: >90% spec: 100% Typical field conditions: sens: 25% - 100% spec: 56% - 100%	<ul style="list-style-type: none"> • Species-specific diagnosis. • Quantification of parasitaemia aids treatment follow-up. 	<ul style="list-style-type: none"> • Requires relatively high degree of training and supervision for reliable results. • Sensitivity and specificity dependent on training and supervision. • Special equipment and supplies needed. • Electricity desirable. • Time-consuming. 	0.03 to 0.08**	(22) (11)
Fluorescent microscopy: • Acridine orange [AO] stained thick blood smears); • Quantitative Buffy Coat (QBC™) - (Becton-Dickinson)	AO: 42% - 93% sens/ 52 - 93% spec QBC: 89% sens/ >95% spec	<ul style="list-style-type: none"> • Results attainable more quickly than normal microscopy. 	<ul style="list-style-type: none"> • Special equipment and supplies needed. • Sensitivity of AO poor with low parasite densities. • Electricity required. • Unreliable species diagnosis; non-specific staining of debris and non-parasitic cells. • QBC will not quantify parasitaemia. • Acridine orange is a hazardous material. 	0.03 (AO) to 1.70 (QBC)	(24) (22)
Clinical, especially based on formal algorithm such as Integrated Management of Childhood Illnesses (IMCI) or similar algorithm	Variable depending on level of clinical competency, training, and malaria risk (endemicity): with IMCI: low risk: sens: 87% spec: 8% high risk: sens: 100% spec: 0%	<ul style="list-style-type: none"> • Speed and ease of use. • No electricity, no special equipment needed beyond normal clinical equipment (thermometer, stethoscope, otoscope, timer). 	<ul style="list-style-type: none"> • Can result in high degree of misdiagnosis and over-treatment for malaria. • Requires close supervision and retraining to maximize reliability. 	Variable depending on situation.	(111) (112)

Table modified from Stennies, 1999, CDC unpublished document.

* Approximate or projected cost given in US dollars per test performed and reflects only cost of expendable materials unless otherwise noted.

** Cost includes salaries of microscopists and expendable supplies; does not include cost of training, supervision, or equipment.

istic option. Clinical diagnosis offers the advantages of ease, speed, and low cost. In areas where malaria is prevalent, clinical diagnosis usually results in all patients with fever and no apparent other cause being treated for malaria. This approach can identify most patients who truly need antimalarial treatment, but it is also likely to misclassify many who do not (15). Over-diagnosis can be considerable and contributes to misuse of antimalarial drugs. Considerable overlap exists between the signs and

symptoms of malaria and other frequent diseases, especially acute lower respiratory tract infection (ALRI), and can greatly increase the frequency of misdiagnosis and mistreatment (16).

Attempts to improve the specificity of clinical diagnosis for malaria by including signs and symptoms other than fever or history of fever have met with only minimal success (17). The Integrated Management of Childhood Illnesses (IMCI) programme defined an algorithm that has been devel-

oped in order to improve diagnosis and treatment of the most common childhood illnesses in areas relying upon relatively unskilled health care workers working without access to laboratories or special equipment. With this algorithm, every febrile child living in a “high-risk” area for malaria should be considered to have, and be treated for, malaria. “High risk” has been defined in IMCI Adaptation Guides as being any situation where as little as 5% of febrile children between the ages of 2 and 59 months are parasitaemic (18), a definition that will likely lead to significant over-diagnosis of malaria in areas with low to moderate malaria transmission.

2.3.3 Antigen detection tests (also known as rapid or “dipstick” tests)

A third diagnostic approach involves the rapid detection of parasite antigens using rapid immunochromatographic techniques. Multiple experimental tests have been developed targeting a variety of parasite antigens (19, 20, 21). A number of commercially available kits (e.g. ParaSight-F®, Becton-Dickinson; Malaquick®, ICT, Sydney, New South Wales, Australia) are based on the detection of the histidine-rich protein 2 (HRP-II) of *P. falciparum*. Compared with light microscopy and QBC, this test yielded rapid and highly sensitive diagnosis of *P. falciparum* infection (22, 23). Advantages to this technology are that no special equipment is required, minimal training is needed, the test and reagents are stable at ambient temperatures, and no electricity is needed. The principal disadvantages are a currently high per-test cost and an inability to quantify the density of infection. Furthermore, for tests based on HRP-II, detectable antigen can persist for days after adequate treatment and cure; therefore, the test cannot adequately distinguish a resolving infection from treatment failure due to drug resistance, especially early after treatment (23). Additionally, a test based on detection of a specific parasite enzyme (lactate dehydrogenase or pLDH) has been developed (OptiMAL®, Flow Inc. Portland, OR, USA) and reportedly only detects viable parasites, which if true, eliminates prolonged periods of false positivity post-treatment (24, 25, 26). Newer generation antigen detection tests are able to distinguish between falciparum and non-falciparum infections, greatly expanding their usefulness in areas where non-falciparum malaria is transmitted frequently.

2.3.4 Molecular tests

Detection of parasite genetic material through polymerase-chain reaction (PCR) techniques is becoming a more frequently used tool in the diagnosis of malaria, as well as the diagnosis and surveillance of drug resistance in malaria. Specific primers have been developed for each of the four species of human malaria. One important use of this new technology is in detecting mixed infections or differentiating between infecting species when microscopic examination is inconclusive (27). In addition, improved PCR techniques could prove useful for conducting molecular epidemiological investigations of malaria clusters or epidemics (28). Primary disadvantages to these methods are overall high cost, high degree of training required, need for special equipment, absolute requirement for electricity, and potential for cross-contamination between samples.

2.3.5 Serology

Techniques also exist for detecting anti-malaria antibodies in serum specimens. Specific serological markers have been identified for each of the four species of human malaria. A positive test generally indicates a past infection. Serology is not useful for diagnosing acute infections because detectable levels of anti-malaria antibodies do not appear until weeks into infection and persist long after parasitaemia has resolved. Moreover, the test is relatively expensive, and not widely available.

2.4 Drugs available for treatment of malaria

There are only a limited number of drugs which can be used to treat or prevent malaria (Table 2). The most widely used are quinine and its derivatives and antifolate combination drugs.

2.4.1 Quinine and related compounds

Quinine, along with its dextroisomer quinidine, has been the drug of last resort for the treatment of malaria, especially severe disease. Chloroquine is a 4-aminoquinoline derivative of quinine first synthesized in 1934 and has since been the most widely used antimalarial drug. Historically, it has been the drug of choice for the treatment of non-severe or uncomplicated malaria and for chemoprophylaxis, although drug resistance has dramatically reduced its usefulness. Amodiaquine is a relatively widely

TABLE 2. ANTIMALARIAL DRUGS FOR UNCOMPLICATED MALARIA

Drug name	Use	Half-life (hours)	Dosing (all <i>per os</i>)		Contra-indications	Cost (US\$)*	Comments
			Adult	Paediatric			
COMBINATION THERAPY							
Mefloquine + Artesunate	• Treatment of non-severe falciparum infections thought to be chloroquine and SP resistant.	M: (14–18 days) Art: 0.5–1.4	15 mg (base)/kg to maximum of 1000 mg mefloquine base on second day of treatment, followed by 10 mg (base)/kg to maximum of 500 mg mefloquine base on 3rd day. 4 mg/kg artesunate daily for 3 days.	15 mg (base)/kg mefloquine on 2nd day of treatment followed by 10 mg/kg mefloquine on 3rd day. 4 mg/kg artesunate daily for 3 days.	See under mefloquine monotherapy.	3.90	<ul style="list-style-type: none"> • Safety of artemisinins & MQ during first trimester of pregnancy not established. • Vomiting after mefloquine can be reduced by administering mefloquine on the second and third day after an initial dose of artesunate. • Artemisinin (10 mg/kg po daily for 3 days) can be substituted for artesunate.
Sulfadoxine/Pyrimethamine + Artesunate	• Treatment of non-severe falciparum infections thought to be chloroquine resistant.	S: 100–200 P: 80–100 Art: 0.5–1.4	25 mg/kg sulfa/1.25 mg/kg pyrimethamine per kg as single dose. Average adult dose: 3 tablets as a single dose (equivalent to 1500 mg sulfa /75 mg pyrimethamine).	By weight: 25 mg/kg sulfa/1.25 mg/kg pyrimethamine per kg as single dose. By age: • < 1 year: 2 tablet • 1–3 years: ¾ tablet • 4–8 years: 1 tablet • 9–14 years: 2 tablets • >14 years: 3 tablets	Known SP allergy.	1.12	<ul style="list-style-type: none"> • This combination has not been evaluated as extensively as MQ + artesunate. • Safety of artemisinin during first trimester of pregnancy not established. • Artemisinin (10 mg/kg po daily for 3 days) can be substituted for artesunate.
Lumefantrine + Artemether Trade name: Co-artem; Riamet	• Treatment of non-severe falciparum infections thought to be chloroquine and SP resistant.	L: 3–6 days Art: 4–11	Semi-immune patients: 4 tablets per dose at 0, 8, 24, and 48 hours (total 16 tablet). Non-immune patients: 4 tablets per dose at 0 and 8 hrs, then twice daily for 2 more days (total 24 tablets)	Tablets per dose by body weight: 10–14 kg: 1 tablet 15–24 kg: 2 tablets 25–34 kg: 3 tablets >34 kg: 4 tablets given in same schedule as for adults, depending on immune status.		7.30 (Cameroon street price); 57 (Europe)	<ul style="list-style-type: none"> • Fixed-dose combination with each tablet containing 20 mg artemether and 120 mg lumefantrine. • Safety during pregnancy not established.
SINGLE-AGENT THERAPY							
Chloroquine (CQ) Trade names: Nivaquine, Malariaquine, Aralen, many others	<ul style="list-style-type: none"> • Treatment of non-falciparum infections. • Treatment of <i>P. falciparum</i> infections in areas where chloroquine remains effective. • Chemoprophylaxis in areas where chloroquine remains effective. 	(41±14 days)	<ul style="list-style-type: none"> • Treatment: 25 mg base/kg divided over 3 days. Average adult: 1 g chloroquine (salt), followed by 500 mg (salt) in 6–8 hours and 500 mg (salt) daily for 2 more days. • Prophylaxis: 500 mg salt once per week. 	<ul style="list-style-type: none"> • Treatment: 25 mg base/kg divided over 3 days. • Prophylaxis: 5 mg base/kg once per week. 		0.08	<ul style="list-style-type: none"> • Widespread resistance in <i>P. falciparum</i> in most regions. • Resistance in <i>P. vivax</i> occurs. • Can cause pruritus in dark-skinned patients, reducing compliance.

TABLE 2 (continued)

Drug name	Use	Half-life (hours)	Dosing (all <i>per os</i>)		Contra-indications	Cost (US\$)*	Comments
			Adult	Paediatric			
SINGLE-AGENT THERAPY							
Amodiaquine (AQ) Trade names: Camoquine, others	• Treatment of non-severe falciparum infections thought to be chloroquine resistant.		• Treatment: 25 mg base/kg divided over 3 days.	• Treatment: 25 mg base/kg divided over 3 days.		0.14	• Cross-resistance with chloroquine limits usefulness in areas with high rates of chloroquine resistance. • Has been associated with toxic hepatitis and agranulocytosis when used as prophylaxis—risk when used for treatment unknown but likely to be low.
Sulfadoxine/pyrimethamine (SP); Sulfalene/pyrimethamine (Metakelfin)	• Treatment of non-severe falciparum infections thought to be chloroquine resistant.	SD: 100–200 SL: 65 P: 80–100	25 mg/kg sulfa/1.25 mg/kg pyrimethamine per kg as single dose. Average adult: 1500 mg sulfa/75 mg pyrimethamine as single dose. (Equivalent to 3 tablets as a single dose.)	By weight: 25 mg/kg sulfa/1.25 mg/kg pyrimethamine per kg as single dose: By age: • < 1 year: 1/2 tablet • 1–3 years: 3/4 tablet • 4–8 years: 1 tablet • 9–14 years: 2 tablets • >14 years: 3 tablets	• Known sulfa allergy	0.12	• Efficacy for vivax infections may be poor. • Widespread resistance in <i>P. falciparum</i> in some regions. • Can cause severe skin disease when used prophylactically; risk when used as treatment unknown but likely to be very low.
Mefloquine (MQ) Trade names: Lariam, Mephaquine	• Treatment of non-severe falciparum infections thought to be chloroquine and SP resistant. • Chemoprophylaxis in areas with chloroquine resistance.	(14–18 days)	• Treatment: 750 mg base to 1500 mg base depending on local resistance patterns. Larger doses (>15 mg/kg) best given in split doses over 2 days. • Prophylaxis: 250 mg once per week.	Treatment: 15 mg (base)/kg to 25 mg (base)/kg depending on local resistance patterns. Larger doses (>15 mg/kg) best given in split doses over 2 days. • Prophylaxis: 5 mg base/kg once per week.	• Known or suspected history of neuro-psychiatric disorder. • history of seizures • concomitant use of halofantrine.	1.92	• Vomiting can be a common problem among young children. • In some populations (e.g. very young African children), unpredictable blood levels, even after appropriate dosing, can produce frequent treatment failure. • Use of lower dose may facilitate development of resistance.
Halofantrine	• Treatment of suspected multidrug-resistant falciparum.	10–90	8 mg base/kg every 6 hours for 3 doses. Average adult: 1500 mg base divided into 3 doses as above.	8 mg base/kg every 6 hours for 3 doses.	• Preexisting cardiac disease. • Congenital prolongation of QT _c interval. • Treatment with mefloquine within prior 3 weeks. • Pregnancy.	5.31	• Cross-resistance with mefloquine has been reported. • Reported to have highly variable bioavailability. • Risk of fatal cardiotoxicity.
Quinine	• Treatment of severe malaria. • Treatment of multidrug-resistant <i>P. falciparum</i> . • Treatment of malaria during 1st trimester of pregnancy.	10–12	• Non-severe malaria: 8 mg (base)/kg 3 times daily for 7 days. • Average adult: 650 mg 3 times per day for 7 days. • Severe: see section on treatment of severe malaria.	• Non-severe malaria: 8 mg (base)/kg 3 times daily for 7 days. • Severe: see section on treatment of severe malaria.		1.51	• Side-effects can greatly reduce compliance. • Used in combination with tetracycline, doxycycline, clindamycin, or SP (where effective) and in areas where quinine resistance not prevalent; duration of quinine dosage can be reduced to 3 days when used in combination.

TABLE 2 (continued)

Drug name	Use	Half-life (hours)	Dosing (all <i>per os</i>)		Contra-indications	Cost (US\$)*	Comments
			Adult	Paediatric			
SINGLE-AGENT THERAPY							
Tetracycline (tetra)/ Doxycycline (doxy)	<ul style="list-style-type: none"> In combination with quinine, can increase efficacy of treatment in areas with quinine resistance and/or reduce likelihood of quinine-associated side-effects by reducing duration of quinine treatment. Prophylaxis. 	Tetra: 10 Doxy: 16	Tetra: 250 mg/kg 4 times per day for 7 days. Doxy: 100 mg/kg 2 times per day for 7 days. Prophylaxis: 100 mg doxy per day.	Tetra: 5 mg/kg 4 times per day for 7 days. Doxy: 2 mg/kg twice per day for 7 days. Prophylaxis: 2 mg/kg doxy per day up to 100 mg.	<ul style="list-style-type: none"> Age less than 8 years. Pregnancy. 		<ul style="list-style-type: none"> Used only in combination with a rapidly acting schizonticide such as quinine.
Clindamycin	<ul style="list-style-type: none"> For patients unable to take tetracycline. In combination with quinine, can increase efficacy of treatment in areas with quinine resistance and/or reduce likelihood of quinine-associated side effects by reducing duration of quinine treatment. 	3	300 mg 4 times per day for 5 days.	20 to 40 mg/kg/day divided in 3 daily doses for 5 days.	<ul style="list-style-type: none"> Severe hepatic or renal impairment. History of gastrointestinal disease, especially colitis. 		<ul style="list-style-type: none"> Is not as effective as tetracycline, especially among non-immune patients. Used only in combination with a rapidly acting schizonticide such as quinine.
Atovaquone/proguanil Trade name: Malarone	<ul style="list-style-type: none"> Treatment of multidrug resistant <i>P. falciparum</i> infections. 	Atv: 59 Prog: 24	1000 mg atovaquone + 400 mg proguanil daily for 3 days.	No pediatric formulation currently available, but for patients between 11 and 40 kg body weight: 11–20 kg: 1/4 adult dose 21–30 kg: 1/2 adult dose 31–40 kg: 3/4 adult dose		35.00	<ul style="list-style-type: none"> Fixed dose combination. Reportedly safe in pregnancy and young children. Drug donation program exists Pediatric formulation in development.
Artemisinin compounds	<ul style="list-style-type: none"> Treatment of multi-drug resistant <i>P. falciparum</i> infections. 	0.5–1.4	4 mg/kg on the first day followed by 2 mg/kg daily for total of 5 to 7 days.	4 mg/kg on the first day followed by 2 mg/kg daily for total of 5 to 7 days.		1.50–3.40	<ul style="list-style-type: none"> Safety for use in pregnancy not fully established, especially for use in first trimester (available data suggest relative safety for second or third trimester). Other artemisinin derivatives include arteether, dihydro-artemisinin, artelinate.
		2–7	20 mg/kg on the first day followed by 10 mg/kg daily for total of 5 to 7 days.	20 mg/kg on the first day followed by 10 mg/kg daily for total of 5 to 7 days.		1.50–3.40	
		4–11	4 mg/kg on the first day followed by 2 mg/kg daily for total of 5 to 7 days.	4 mg/kg on the first day followed by 2 mg/kg daily for total of 5 to 7 days.		3.60–4.80	
Primaquine	<ul style="list-style-type: none"> Treatment of <i>P. vivax</i> infections (reduce likelihood of relapse). Gametocytocidal agent. 	6	<ul style="list-style-type: none"> 14 mg base per day for 14 days. 45 mg once per week for 8 weeks. 	0.3 mg (base)/kg daily for 14 days.	<ul style="list-style-type: none"> G6PD deficiency. Pregnancy. 		<ul style="list-style-type: none"> Primaquine has also been investigated for prophylaxis use. Shorter courses have been used for falciparum infections for gametocytocidal action.

* Cost is given for a full adult (60kg) treatment course. Prices have been derived from a variety of sources including Management Sciences for Health, World Health Organization, drug companies, published reports, and personal communication and are presented for relative comparison purposes only—actual local prices may differ greatly.

available compound closely related to chloroquine. Other quinine-related compounds in common use include primaquine (specifically used for eliminating the exoerythrocytic forms of *P. vivax* and *P. ovale* that cause relapses), and mefloquine (a quinoline-methanol derivative of quinine).

2.4.2 Antifolate combination drugs

These drugs are various combinations of dihydrofolate-reductase inhibitors (proguanil, chlorproguanil, pyrimethamine, and trimethoprim) and sulfa drugs (dapson, sulfalene, sulfamethoxazole, sulfadoxine, and others). Although these drugs have antimalarial activity when used alone, parasitological resistance can develop rapidly. When used in combination, they produce a synergistic effect on the parasite and can be effective even in the presence of resistance to the individual components. Typical combinations include sulfadoxine/pyrimethamine (SP or Fansidar¹), sulfalene-pyrimethamine (metakelfin), and sulfamethoxazole-trimethoprim (co-trimoxazole).

A new antifolate combination drug is currently being tested in Africa. This drug, a combination of chlorproguanil and dapson, also known as Lap-Dap, has a much more potent synergistic effect on malaria than existing drugs such as SP. Benefits of this combination include 1) a greater cure rate, even in areas currently experiencing some level of SP resistance, 2) a lower likelihood of resistance developing because of a more advantageous pharmacokinetic and pharmacodynamic profile, and 3) probable low cost (currently estimated at less than US\$ 1 per adult treatment course) (29).

2.4.3 Antibiotics

Tetracycline and derivatives such as doxycycline are very potent antimalarials and are used for both treatment and prophylaxis. In areas where response to quinine has deteriorated, tetracyclines are often used in combination with quinine to improve cure rates. Clindamycin has been used but offers only limited advantage when compared to other available antimalarial drugs. Parasitological response is slow to clindamycin and recrudescence rates are high (30, 31). Its efficacy among non-immune individuals has not been fully established.

2.4.4 Artemisinin compounds

A number of sesquiterpene lactone compounds have been synthesized from the plant *Artemisia annua* (artesunate, artemether, arteether). These compounds are used for treatment of severe malaria and have shown very rapid parasite clearance times and faster fever resolution than occurs with quinine. In some areas of South-East Asia, combinations of artemisinins and mefloquine offer the only reliable treatment for even uncomplicated malaria, due to the development and prevalence of multidrug-resistant falciparum malaria (32). Combination therapy (an artemisinin compound given in combination with another antimalarial, typically a long half-life drug like mefloquine) has reportedly been responsible for inhibiting intensification of drug resistance and for decreased malaria transmission levels in South-East Asia (32, 33) (see section 6.1.3).

2.4.5 Miscellaneous compounds (not exhaustive)

Halofantrine is a phenanthrene-methanol compound with activity against the erythrocytic stages of the malaria parasite. Its use has been especially recommended in areas with multiple drug-resistant falciparum. Recent studies have indicated, however, that the drug can produce potentially fatal cardiac conduction abnormalities (specifically, prolongation of the PR and QT interval), limiting its usefulness (34). Atovaquone is a hydroxynaphthoquinone that is currently being used most widely for the treatment of opportunistic infections in immunosuppressed patients. It is effective against chloroquine-resistant *P. falciparum*, but because, when used alone, resistance develops rapidly, atovaquone is usually given in combination with proguanil (35, 36). A new fixed dose antimalarial combination of 250 mg atovaquone and 100 mg proguanil (MalaroneTM) is being brought to market worldwide and is additionally being distributed through a donation programme (37, 38). Two drugs originally synthesized in China are currently undergoing field trials. Pyronaridine was reportedly 100% effective in one trial in Cameroon (39); however, it was only between 63% and 88% effective in Thailand (40). Lumefantrine, a fluoromethanol compound, is being produced as a fixed combination tablet with artemether (41, 42).

¹ Note: Use of trade names is for identification only and does not imply endorsement by the Public Health Service or by the US Department of Health and Human Services.

2.4.6 Combination therapy with antimalarials

The use of two antimalarials simultaneously, especially when the antimalarials have different mechanisms of action, has the potential for inhibiting the development of resistance to either of the components. The efficacy of a combination of a 4-aminoquinoline drug (either chloroquine or amodiaquine) with sulfadoxine/pyrimethamine has been reviewed (43). The results of this review suggested that the addition of either chloroquine or amodiaquine to SP marginally improved parasitological clearance (compared with SP alone). The studies reviewed were mostly done in areas and at times when both SP and chloroquine/amodiaquine retained a fair amount of efficacy, and it is not clear from these studies how well such a combination would act in areas where one of the components was significantly compromised. Additionally, to date, there are no data to suggest whether this slightly improved clearance would

translate into prolonged useful life span for either drug.

Another combination therapy approach, combining an artemisinin derivative with other, longer half-life antimalarials, is discussed in section 6.1.3.

2.5 Current status of drug-resistant malaria

Resistance to antimalarial drugs has been described for two of the four species of malaria parasite that naturally infect humans, *P. falciparum* and *P. vivax*. *P. falciparum* has developed resistance to nearly all antimalarials in current use, although the geographical distribution of resistance to any single antimalarial drug varies greatly (Table 3). *P. vivax* infection acquired in some areas has been shown to be resistant to chloroquine and/or primaquine (44, 45).

Chloroquine-resistant *P. falciparum* malaria has

TABLE 3. DISTRIBUTION OF DRUG-RESISTANT *PLASMODIUM FALCIPARUM* MALARIA

Region	Resistance reported ¹			Comments	
	CQ	SP	MQ	Others	
PLASMODIUM FALCIPARUM INFECTIONS					
Central America (Mexico, Belize, Guatemala, Honduras, El Salvador, Nicaragua, Costa Rica, NW Panama)	N	N	N		North-west of Panama Canal only
Caribbean (Haiti and Dominican Republic only)	N	N	N		
South America (SE Panama, Columbia, Venezuela, Ecuador, Peru, Brazil, Bolivia,)	Y	Y	Y	QN	Resistance to MQ and QN, although reported, is considered to occur infrequently
Western Africa	Y	Y	Y		Incidence of resistance to CQ variable, but very common in most areas
Eastern Africa	Y	Y	N		Incidence of resistance to SP highly variable, with some reports of focally high incidence, but generally uncommon
Southern Africa	Y	Y	N		Resistance to SP, although reported, is considered to be generally uncommon
Indian Subcontinent	Y	N	N		
South-East Asia and Oceania	Y	Y	Y	HAL, QN	Border areas of Thailand, Cambodia, and Myanmar highest risk for multiple-drug-resistant infections; in other areas, incidence of resistance to SP and MQ highly variable and absent in many areas
East Asia (China)	Y	Y	?		Resistance greatest problem in southern China

¹ Reports of resistance to a given agent occurring in an area does not necessarily mean that occurrence is frequent enough to pose a significant public health risk. Bold "Y" indicates agents to which significant resistance occurs in a given area (although actual risk may be highly focal, e.g. South-East Asia, where MQ resistance, while very frequent in some limited areas, is infrequent or absent in most others). Regular "Y" indicates that, while resistance to agent has been reported, it is not believed to occur frequently enough to pose a significant public health risk.

CQ = chloroquine QN = quinine SP = sulfadoxine-pyrimethamine HAL = halofantrine MQ = mefloquine

been described everywhere that *P. falciparum* malaria is transmitted except for malarious areas of Central America (north-west of the Panama Canal), the island of Hispaniola, and limited areas of the Middle East and Central Asia. Sulfadoxine-pyrimethamine (SP) resistance occurs frequently in South-East Asia and South America. SP resistance is becoming more prevalent in Africa as that drug

is increasingly being relied upon as a replacement for chloroquine. Mefloquine resistance is frequent in some areas of South-East Asia and has been reported in the Amazon region of South America and sporadically in Africa (46). Cross-resistance between halofantrine and mefloquine is suggested by reduced response to halofantrine when used to treat mefloquine failures (47).

3. Causes of resistance

3.1 Definition of antimalarial drug resistance

Antimalarial drug resistance has been defined as 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 tolerance of the subject”. This definition was later modified to specify that the drug in question must “gain access to the parasite or the infected red blood cell for the duration of the time necessary for its normal action” (48). Most researchers interpret this as referring only to persistence of parasites after treatment doses of an antimalarial rather than prophylaxis failure, although the latter is a useful tool for early warning of the presence of drug resistance (49).

This definition of resistance requires demonstration of malaria parasitaemia in a patient who has received an observed treatment dose of an antimalarial drug and simultaneous demonstration of adequate blood drug and metabolite concentrations using established laboratory methods (such as high performance liquid chromatography) or *in vitro* tests (see section 4.2). In practice, this is rarely done with *in vivo* studies. *In vivo* studies of drugs for which true resistance is well known (such as chloroquine) infrequently include confirmation of drug absorption and metabolism; demonstration of persistence of parasites in a patient receiving directly observed therapy is usually considered sufficient. Some drugs, such as mefloquine, are known to produce widely varying blood levels after appropriate dosing and apparent resistance can often be explained by inadequate blood levels (50).

3.2 Malaria treatment failure

A distinction must be made between a failure to clear malarial parasitaemia or resolve clinical disease following a treatment with an antimalarial drug and true antimalarial drug resistance. While drug resistance can cause treatment failure, not all treatment failure is due to drug resistance. Many factors can contribute to treatment failure including

incorrect dosing, non-compliance with duration of dosing regimen, poor drug quality, drug interactions, poor or erratic absorption, and misdiagnosis. Probably all of these factors, while causing treatment failure (or apparent treatment failure) in the individual, may also contribute to the development and intensification of true drug resistance through increasing the likelihood of exposure of parasites to suboptimal drug levels.

3.3 Mechanisms of antimalarial resistance

In general, resistance appears to occur through spontaneous mutations that confer reduced sensitivity to a given drug or class of drugs. For some drugs, only a single point mutation is required to confer resistance, while for other drugs, multiple mutations appear to be required. Provided the mutations are not deleterious to the survival or reproduction of the parasite, drug pressure will remove susceptible parasites while resistant parasites survive. Single malaria isolates have been found to be made up of heterogeneous populations of parasites that can have widely varying drug response characteristics, from highly resistant to completely sensitive (51). Similarly, within a geographical area, malaria infections demonstrate a range of drug susceptibility. Over time, resistance becomes established in the population and can be very stable, persisting long after specific drug pressure is removed.

The biochemical mechanism of resistance has been well described for chloroquine, the antifolate combination drugs, and atovaquone.

3.3.1 Chloroquine resistance

As the malaria parasite digests haemoglobin, large amounts of a toxic by-product are formed. The parasite polymerizes this by-product in its food vacuole, producing non-toxic haemozoin (malaria pigment). It is believed that resistance of *P. falciparum* to chloroquine is related to an increased capacity for the parasite to expel chloroquine at a rate that does not allow chloroquine to

reach levels required for inhibition of haem polymerization (52). This chloroquine efflux occurs at a rate of 40 to 50 times faster among resistant parasites than sensitive ones (53). Further evidence supporting this mechanism is provided by the fact that chloroquine resistance can be reversed by drugs which interfere with this efflux system (54). It is unclear whether parasite resistance to other quinoline antimalarials (amodiaquine, mefloquine, halofantrine, and quinine) occurs via similar mechanisms (52).

3.3.2 Antifolate combination drugs

Antifolate combination drugs, such as sulfadoxine + pyrimethamine, act through sequential and synergistic blockade of 2 key enzymes involved with folate synthesis. Pyrimethamine and related compounds inhibit the step mediated by dihydrofolate reductase (DHFR) while sulfones and sulfonamides inhibit the step mediated by dihydropteroate synthase (DHPS) (48). Specific gene mutations encoding for resistance to both DHPS and DHFR have been identified. Specific combinations of these mutations have been associated with varying degrees of resistance to antifolate combination drugs (55).

3.3.3 Atovaquone

Atovaquone acts through inhibition of electron transport at the cytochrome *bc1* complex (56). Although resistance to atovaquone develops very rapidly when used alone, when combined with a second drug, such as proguanil (the combination used in Malarone™) or tetracycline, resistance develops more slowly (35). Resistance is conferred by single-point mutations in the cytochrome-b gene.

3.4 Factors contributing to the spread of resistance

Numerous factors contributing to the advent, spread, and intensification of drug resistance exist, although their relative contribution to resistance is unknown. Factors that have been associated with antimalarial drug resistance include such disparate issues as human behaviour (dealt with in detail elsewhere), vector and parasite biology, pharmacokinetics, and economics. As mentioned previously, conditions leading to malaria treatment failure may also contribute to the development of resistance.

3.4.1 Biological influences on resistance

Based on data on the response of sensitive parasites to antimalarial drugs *in vitro* and the pharmacokinetic profiles of common antimalarial drugs, there is thought to always be a residuum of parasites that are able to survive treatment (57). Under normal circumstances, these parasites are removed by the immune system (non-specifically in the case of non-immune individuals). Factors that decrease the effectiveness of the immune system in clearing parasite residuum after treatment also appear to increase survivorship of parasites and facilitate development and intensification of resistance. This mechanism has been suggested as a significant contributor to resistance in South-East Asia, where parasites are repeatedly cycled through populations of non-immune individuals (58, 59); the non-specific immune response of non-immune individuals is less effective at clearing parasite residuum than the specific immune response of semi-immune individuals (60). The same mechanism may also explain poorer treatment response among young children and pregnant women (60).

The contribution to development and intensification of resistance of other prevalent immunosuppressive states has not been evaluated. Among refugee children in the former Zaire, those who were malnourished (low weight for height) had significantly poorer parasitological response to both chloroquine and SP treatment (61). Similarly, evidence from prevention of malaria during pregnancy suggests that parasitological response to treatment among individuals infected with the human immunodeficiency virus (HIV) may also be poor. HIV-seropositive women require more frequent treatment with SP during pregnancy in order to have the same risk of placental malaria as is seen among HIV-seronegative women (62). Parasitological response to treatment of acute malaria among HIV-seropositive individuals has not been evaluated. The current prevalence of malnutrition among African children under 5 years has been estimated to be 30% and an estimated 4 to 5 million children are expected to be infected with HIV at the beginning of this new century (63). If it is proven that malnutrition or HIV infection plays a significant role in facilitating the development or intensification of antimalarial drug resistance, the prevalence of these illnesses could pose a tremendous threat to existing and future antimalarial drugs.

Some characteristics of recrudescence or drug-resistant infections appear to provide a survival

advantage or to facilitate the spread of resistance conferring genes in a population (32). In one study, patients experiencing chloroquine treatment failure had recrudescence infections that tended to be less severe or even asymptomatic (64). Schizont maturation may also be more efficient among resistant parasites (65, 66).

There is some evidence that certain combinations of drug-resistant parasites and vector species enhance transmission of drug resistance, while other combinations inhibit transmission of resistant parasites. In South-East Asia, two important vectors, *Anopheles stephensi* and *A. dirus*, appear to be more susceptible to drug-resistant malaria than to drug-sensitive malaria (67, 68). In Sri Lanka, researchers found that patients with chloroquine-resistant malaria infections were more likely to have gametocytaemia than those with sensitive infections and that the gametocytes from resistant infections were more infective to mosquitoes (64). The reverse is also true—some malaria vectors may be somewhat refractory to drug-resistant malaria, which may partially explain the pockets of chloroquine sensitivity that remain in the world in spite of very similar human populations and drug pressure (e.g. Haiti).

Many antimalarial drugs in current usage are closely related chemically and development of resistance to one can facilitate development of resistance to others. Chloroquine and amodiaquine are both 4-aminoquinolines and cross-resistance between these two drugs is well known (69, 70). Development of resistance to mefloquine may also lead to resistance to halofantrine and quinine. Antifolate combination drugs have similar action and widespread use of sulfadoxine/ pyrimethamine for the treatment of malaria may lead to increased parasitological resistance to other antifolate combination drugs (29). Development of high levels of SP resistance through continued accumulation of DHFR mutations may compromise the useful life span of newer antifolate combination drugs such as chlorproguanil/dapsone (LapDap) even before they are brought into use. This increased risk of resistance due to SP use may even affect non-malarial pathogens; use of SP for treatment of malaria increased resistance to trimethoprim/sulfamethoxazole among respiratory pathogens (71).

There is an interesting theory that development of resistance to a number of antimalarial drugs among some falciparum parasites produces a level of genetic plasticity that allows the parasite to rapidly adapt to a new drug, even when the new

drug is not chemically related to drugs previously experienced (72). The underlying mechanism of this plasticity is currently unknown, but this capacity may help explain the rapidity with which South-East Asian strains of falciparum develop resistance to new antimalarial drugs.

The choice of using a long half-life drug (SP, MQ) in preference to one with a short half-life (CQ, LapDap, QN) has the benefit of simpler, single-dose regimens which can greatly improve compliance or make directly observed therapy feasible. Unfortunately, that same property may increase the likelihood of resistance developing due to prolonged elimination periods. The relative contribution of low compliance versus use of long half-life drugs to development of resistance is not known.

Parasites from new infections or recrudescence parasites from infections that did not fully clear will be exposed to drug blood levels that are high enough to exert selective pressure but are insufficient to provide prophylactic or suppressive protection (57). When blood levels drop below the minimum inhibitory concentration (the level of drug that fully inhibits parasite growth), but remain above the EC₅₀ (the concentration of drug that produces 5% inhibition of parasite growth), selection of resistant parasites occurs. This selection was illustrated in one study in Kenya that monitored drug sensitivity of parasites reappearing after SP treatment. Parasites reappearing during a period when blood levels were below the point required to clear pyrimethamine-resistant parasites, but still above that level required to clear pyrimethamine-sensitive parasites, were more likely to be pyrimethamine-resistant than those reappearing after levels had dropped below the level required to clear pyrimethamine-sensitive parasites (73). This period of selective pressure lasts for approximately one month for mefloquine, whereas it is only 48 hours for quinine (57).

In areas of high malaria transmission, the probability of exposure of parasites to drug during this period of selective pressure is high. In Africa, for instance, people can be exposed to as many as 300 infective bites per year (in rare cases, even as much as 1000 infective bites per year), and during peak transmission, as many as five infective bites per night (74, 75).

Mismatched pharmacokinetics can also play a role in facilitating the development of resistance. The elimination half-life of pyrimethamine is between 80 and 100 hours, and is between 100 and 200 hours for sulfadoxine, leaving an extended period when sulfadoxine is “unprotected” by

synergy with pyrimethamine (73). This sort of mismatched pharmacokinetics is even more apparent in the mefloquine-sulfadoxine-pyrimethamine (MSP) combination used in Thailand (mefloquine has an elimination half-life of approximately 336 to 432 hours (48, 76).

It is not clear what the relationship between transmission intensity and development of resistance is, although most researchers agree that there seems to be such an association. It is apparent that there are more genetically distinct clones per person in areas of more intense transmission than in areas of lower transmission (77). However, the interpretation of this and its implications for development of resistance has variously been described as resistance being more likely in low-transmission environments (78, 79), high-transmission environments (80, 77, 81), or either low- or high- but not intermediate-transmission environments (82, 83). This relationship between transmission intensity and parasite genetic structure is obviously complex and subject to other confounding/contributing factors (84, 83). What is clear is that the rate at which resistance develops in a given area is sensitive to a number of factors beyond mere intensity of transmission (such as initial prevalence of mutations, intensity of drug pressure, population movement between areas, the nature of acquired immunity to the parasite or its strains, etc.), but that reducing the intensity of transmission will likely facilitate prolonging the useful life span of drugs (81, 85).

3.4.2 Programmatic influences on resistance

Programmatic influences on development of antimalarial drug resistance include overall drug pressure, inadequate drug intake (poor compliance or inappropriate dosing regimens), pharmacokinetic and pharmacodynamic properties of the drug or drug combination, and drug interactions (86). Additionally, reliance on presumptive treatment can facilitate the development of antimalarial drug resistance.

Overall drug pressure, especially that exerted by programmes utilizing mass drug administration, probably has the greatest impact on development of resistance (86, 87). Studies have suggested that resistance rates are higher in urban and periurban areas than rural communities, where access to and use of drug is greater (88).

Confusion over proper dosing regimen has been described. In Thailand, the malaria control pro-

gramme recommended 2 tablets (adult dose) of SP for treating malaria based on studies suggesting that this was effective. Within a few years, this was no longer effective and the programme increased the regimen to 3 tablets. Although unproven, this may have contributed to the rapid loss of SP efficacy there. Similar confusion over the proper SP dosing regimen exists in Africa. To simplify treatment, many programmes dose children based on age rather than weight and, depending on the regimen being recommended, this has been shown to produce systematic underdosing among children of certain weight and age groups (ter Kuile, unpublished data, 1997).

The use of presumptive treatment for malaria has the potential for facilitating resistance by greatly increasing the number of people who are treated unnecessarily but will still be exerting selective pressure on the circulating parasite population. In some areas and at some times of the year, the number of patients being treated unnecessarily for malaria can be very large (15).

Concurrent treatment with other drugs can increase the likelihood of treatment failure and may contribute to development of drug resistance. Folate administration for treatment of anaemia and possibly when used as a routine supplement during pregnancy (CDC, unpublished data, 1998), can increase treatment failure rates (89). Similarly, concurrent illness may have an influence, as was mentioned previously with regard to malnourishment.

Drug quality has also been implicated in ineffective treatment and possibly drug resistance. Either through poor manufacturing practices, intentional counterfeiting, or deterioration due to inadequate handling and storage, drugs may not contain sufficient quantities of the active ingredients. In an analysis of chloroquine and antibiotics available in Nigeria and Thailand, between 37% and 40% of samples assayed had substandard content of active ingredients, mostly from poor manufacturing practices (90). Another study in Africa found chloroquine stored under realistic tropical conditions lost at least 10% of its activity in a little over a year (91).

4. Detection of resistance

In general, four basic methods have been routinely used to study or measure antimalarial drug resistance: *in vivo*, *in vitro*, animal model studies, and molecular characterization. Additionally, less rigorous methods have been used, such as case reports, case series, or passive surveillance. Much discussion has occurred regarding the relative merits of one test over another, with the implication always being that one type of test should be used preferentially. Careful consideration of the types of information each yields should indicate, however, that these are complementary, rather than competing, sources of information about resistance.

Recognition of drug resistance (or, more appropriately, treatment failure) in individual patients is made difficult in many settings by operational issues, such as availability and quality of microscopy. Especially in Africa, where presumptive diagnosis and treatment for malaria is the rule, detection of treatment failures also tends to be presumptive (persistence or reappearance of clinical symptoms in a patient recently receiving malaria treatment). Because of the non-specific nature of clinical signs and symptoms of malaria and the many other causes of febrile disease, this can lead to a false sense that a particular drug is not working when it is, or, potentially, that an ineffective drug is working when it is not. In cases where microscopy is used, presence of parasitaemia in a supposedly fully treated patient may indicate treatment failure, but is not necessarily evidence of drug resistance, as explained in section 3.1.

4.1 *In vivo* tests

An *in vivo* test consists of the treatment of a group of symptomatic and parasitaemic individuals with known doses of drug and the subsequent monitoring of the parasitological and/or clinical response over time. One of the key characteristics of *in vivo* tests is the interplay between host and parasite. Diminished therapeutic efficacy of a drug can be masked by immune clearance of parasites among patients with a high degree of acquired immunity (60).

Of the available tests, *in vivo* tests most closely reflect actual clinical or epidemiological situations (i.e. the therapeutic response of currently circulating parasites infecting the actual population in which the drug will be used). Because of the influence of external factors (host immunity, variations of drug absorption and metabolism, and potential misclassification of reinfections as recrudescences), the results of *in vivo* tests do not necessarily reflect the true level of pure antimalarial drug resistance. However, this test offers the best information on the efficacy of antimalarial treatment under close to actual operational conditions—what can be expected to occur among clinic patients if provider and patient compliance is high.

The original methods for *in vivo* tests required prolonged periods of follow-up (minimum of 28 days) and seclusion of patients in screened rooms to prevent the possibility of reinfection. These methods have since been modified extensively and the most widely used methods now involve shorter periods of follow-up (7 to 14 days) without seclusion, under the assumption that reappearance of parasites within 14 days of treatment is more likely due to recrudescence than reinfection (92). Additional modifications reflect the increased emphasis on clinical response in addition to parasitological response. Traditionally, response to treatment was categorized purely on parasitological grounds as Sensitive, RI, RII, and RIII (48). Later modifications have combined, to varying extent, parasitological and clinical indicators (93).

Because anaemia can be a major component of malaria illness, *in vivo* methodologies allow investigation of haematological recovery after malaria therapy (94). This is obviously not possible with *in vitro* or molecular techniques. Failure of complete parasitological clearance, even in situations where recurrence of fever is rare, can be associated with lack of optimal haematological recovery among anaemic patients.

Unfortunately, these methodologies, while termed “standardized” are, in practice, not standardized. Major differences in sample size, enrolment

criteria, exclusion criteria, length and intensity of follow-up, loss-to-follow-up rates, and interpretation and reporting of results are apparent in published papers on *in vivo* trials. These differences are at times so dramatic, that it is difficult, if not impossible, to compare results from one study to another with any level of confidence (CDC, unpublished data, 1999).

The methodology currently being used and promoted, especially in sub-Saharan Africa, is a system that emphasizes clinical response over parasitological response (95). Close adherence to this protocol does provide comparable data; however, these data are not readily comparable to data collected using other *in vivo* methods. Although not called for in the protocol, categorization of the parasitological response using the standard WHO definitions (95) would allow some ability to compare to historical levels and provide useful parasitological results that would aid in interpreting the clinical results.

4.2 *In vitro* tests

From the point of view of a researcher interested in pure drug resistance, *in vitro* tests avoid many of the confounding factors which influence *in vivo* tests by removing parasites from the host and placing them into a controlled experimental environment. In the most frequently used procedure, the micro-technique, parasites obtained from a finger-prick blood sample are exposed in microtitre plates to precisely known quantities of drug and observed for inhibition of maturation into schizonts (96).

This test more accurately reflects "pure" antimalarial drug resistance. Multiple tests can be performed on isolates, several drugs can be assessed simultaneously, and experimental drugs can be tested. However, the test has certain significant disadvantages. The correlation of *in vitro* response with clinical response in patients is neither clear nor consistent, and the correlation appears to depend on the level of acquired immunity within the population being tested. Prodrugs, such as proguanil, which require host conversion into active metabolites cannot be tested. Neither can drugs that require some level of synergism with the host's immune system. Although adaptation of erythrocytic forms of *P. vivax* to continuous culture has been achieved, non-falciparum erythrocytic parasites generally cannot be evaluated *in vitro* (97). In addition, because parasites must be cultured, dif-

ferential die-off of parasites can occur. If, for instance, resistant strains are less likely to adapt, the results would be biased towards sensitive responses. Because venous blood is typically needed, resistance in the more vulnerable younger age groups is often not studied. Finally, these tests are technologically more demanding and relatively expensive, which makes them potentially more difficult to adapt successfully to routine work in the field.

4.3 Animal model studies

This type of test is, in essence, an *in vivo* test conducted in a non-human animal model and, therefore, is influenced by many of the same extrinsic factors as *in vivo* tests. The influence of host immunity is minimized by using lab-reared animals or animal-parasite combinations unlikely to occur in nature, although other host factors would still be present. These tests allow for the testing of parasites which cannot be adapted to *in vitro* environments (provided a suitable animal host is available) and the testing of experimental drugs not yet approved for use in humans. A significant disadvantage is that only parasites that can grow in, or are adaptable to, non-human primates can be investigated.

4.4 Molecular techniques

These tests are in the process of being developed and validated, but offer promising advantages to the methods described above. Molecular tests use polymerase chain reaction (PCR) to indicate the presence of mutations encoding biological resistance to antimalarial drugs (98). Theoretically, the frequency of occurrence of specific gene mutations within a sample of parasites obtained from patients from a given area could provide an indication of the frequency of drug resistance in that area analogous to information derived from *in vitro* methods. Advantages include the need for only small amounts of genetic material as opposed to live parasites, independence from host and environmental factors, and the ability to conduct large numbers of tests in a relatively short period of time. Disadvantages include the obvious need for sophisticated equipment and training, and the fact that gene mutations that confer antimalarial drug resistance are currently known or debated for only a limited number of drugs (primarily for dihydrofolate reductase inhibitors [pyrimethamine], dihydropteroate synthase inhibitors [sulfadoxine], and chloroquine) (98, 99). Confirmation of the asso-

ciation between given mutations and actual drug resistance is difficult, especially when resistance involves more than one gene locus and multiple mutations. If these complexities can be resolved, molecular techniques may become an extremely valuable surveillance tool for monitoring the occurrence, spread, or intensification of drug resistance.

4.5 Case reports and passive detection of treatment failure

Additional methods for identifying or monitoring antimalarial drug resistance include the use of case reports or case series of spontaneously reported treatment failure. In general, these methods require far less investment in time, money, and personnel and can be done on an ongoing basis by individual health care centres. They suffer, however, from presenting a potentially biased view of drug resistance primarily because denominators are typically unknown and rates of resistance cannot be calculated. Nonetheless, case reports can be useful and may indicate a problem that should be confirmed using one of the other methods. In the United States, for instance, case reports, especially when occurring in clusters, of prophylaxis failure have been used to

help formulate recommendations for chemoprophylaxis of non-immune travellers to endemic areas (100).

Another method that has been used is passive detection of treatment failure. In this system, patients are treated following usual treatment guidelines and told to come back to the clinic or hospital if symptoms persist or return. Those cases which do return are considered to represent the population of treatment failures. Because this system does not ensure compliance with treatment regimens through directly observed therapy and does not attempt to locate and determine the outcome of patients who do not return on their own, data are seriously biased. In one study conducted in Ethiopia and Eritrea using this method, only 1706/39 824 (4.6%) patients returned to clinic (101). The assumption was that those patients who did not return did not have resistant parasites, yielding a very low prevalence of resistance (1.8% to 4.8%, depending on region). These results contrast dramatically with results from standard 7-day *in vivo* trials conducted at two sites in Eritrea in 1994 (CDC, unpublished data, 1994) and one site in Ethiopia in 1993–1994 which found between 58% and 86% RII/RIII level resistance (102).

5. Treatment

In theory, recommended treatment regimens should be tailored specifically to a given region based on resistance patterns found in that area. Other considerations include cost, cost-effectiveness, availability, ease of administration, capabilities of the health care infrastructure (i.e. do health care workers have the equipment and training to safely use parenteral routes of administration?), perceived efficacy, and real and perceived safety of the drug (acceptability of the drug by the population). In practice, currently recommended treatment regimens often do not reflect the current state of antimalarial drug resistance.

Chloroquine remains the drug of choice for treatment of non-falciparum infections and non-severe falciparum infections acquired in areas of known chloroquine sensitivity. Because drug resistance is not an all-or-nothing phenomenon, chloroquine still retains adequate efficacy even in areas of known resistance for continued use to be justifiable for the time being (for instance, some areas of West Africa continue to use chloroquine successfully, although efficacy rates are declining). Much of Africa, however, is currently investigating alternatives to chloroquine. Malawi, Kenya, South Africa, and Botswana have moved away from chloroquine and are using sulfadoxine/ pyrimethamine (SP) extensively or exclusively for treatment of non-severe falciparum infections. The United Republic of Tanzania recently indicated that it is moving towards SP as first-line treatment of malaria, and Ethiopia, Eritrea, Uganda, and others are in the process of considering similar policy changes.

Multidrug resistance (typically referring to resistance to both chloroquine and SP, but may also include resistance to other compounds as well) occurs frequently in Amazonia and South-East Asia. In these areas, a wide range of treatment options are used. Quinine, either alone or in combination with tetracyclines, or mefloquine tend to be the drugs of choice for multidrug-resistant malaria, although declining quinine efficacy and high rates of mefloquine resistance have been reported in some areas of South-East Asia. In limited areas of Thailand, where falciparum is resistant to many of the available drugs, a combination of high-dose mefloquine (25 mg/kg in a divided dose) and artesunate (4 mg/kg daily for 3 days) or 7 days of artesunate alone is required to achieve reliable clearance of parasites.

Table 2 summarizes various treatment options, not all of which would be available or necessarily appropriate in all contexts. One of the primary limiting factors to the use of a highly effective antimalarial and a willingness to change policy to facilitate its use, is the cost of the drug itself. Although a number of evaluations have been able to show the cost-effectiveness of changing between certain drugs, in many cases, the total cost associated with use of a given drug may be prohibitively high (103). Additional costs of interventions to improve use of drugs or patients' adherence to treatment regimens (such as provider and user training, innovative packaging) would further add to the total cost of using some drugs or drug combinations.

6. The future: prevention of drug resistance

The future of antimalarial drug resistance and efforts to combat it is defined by a number of assumptions. First, antimalarial drugs will continue to be needed long into the future. No strategy in existence or in development, short of an unforeseen scientific breakthrough or complete eradication, is likely to be 100% effective in preventing malaria infection. Second, as long as drugs are used, the chance of resistance developing to those drugs is present. *P. falciparum* has developed resistance to nearly all available antimalarial drugs and it is highly likely that the parasite will eventually develop resistance to any drug that is used widely. The advent of *P. vivax* resistant to chloroquine and primaquine may, in time, result in a resurgence of vivax malaria as has been seen with *P. falciparum*. Third, development of new drugs appears to be taking longer than development of parasitological resistance. The development of resistance to antimalarial drugs in South-East Asia has been far quicker than the estimated 12 to 17 years it takes to develop and market a new antimalarial compound (2). Fourth, affordability is an essential consideration for any strategy to control drug-resistant malaria, especially in Africa.

The future, especially in Africa, will also be defined by how well the central tenets of malaria control can be reconciled with the central tenets of control of drug resistance. One of the cornerstones of the current approach to malaria control is the provision of prompt, effective malaria treatment. In much of Africa, easy access to public sector health care is limited and when it is accessible, health care staff are often inadequately trained, insufficiently supplied and supported, ineffectively supervised and/or poorly motivated. One response to this situation has been the intentional liberalization of access to drugs; instead of relying so heavily on the formal public sector to distribute antimalarial drugs, some people are suggesting that the best way to reduce the time between onset of illness and first treatment with an antimalarial drug is by making these drugs widely available on the open market, from unofficial sources of health care, and at the

household level (104). This approach is gaining support internationally. This approach is also in direct conflict with the primary methods for inhibiting development of drug resistance, limited access to and judicious use of chemotherapeutic agents. Clearly, some middle ground will need to be identified that will improve access to antimalarial drugs for those who need to be treated while at the same time reducing the inappropriate use of those same drugs.

Prevention strategies can be divided into those aimed specifically at preventing malaria infection and those aimed at reducing the likelihood of development of drug resistance. Reduction of overall malaria infection rates or transmission rates have an indirect impact on development of drug resistance by reducing the number of infections needing to be treated (and therefore, overall drug pressure) and by reducing the likelihood that resistant parasites are successfully transmitted to new hosts. Full discussion of these strategies is beyond the scope of this review but they include the use of insecticide-treated bednets, indoor residual insecticide spraying, environmental control (mosquito breeding site or "source" reduction), other personal protection measures (e.g. use of repellent soap or screening windows) and chemoprophylaxis in defined populations (use of mass prophylaxis is typically not recommended). An effective and deliverable vaccine would also be greatly beneficial.

6.1 Preventing drug resistance

Interventions aimed at preventing drug resistance, per se, generally focus on reducing overall drug pressure through more selective use of drugs; improving the way drugs are used through improving prescribing, follow-up practices, and patient compliance; or using drugs or drug combinations which are inherently less likely to foster resistance or have properties that do not facilitate development or spread of resistant parasites.

6.1.1 Reducing overall drug pressure.

Because overall drug pressure is thought to be the single most important factor in development of resistance, following more restrictive drug use and prescribing practices would be helpful, if not essential, for limiting the advent, spread, and intensification of drug resistance. This approach has gained support in North America and Europe for fighting antibacterial drug resistance (105, 106).

The greatest decrease in antimalarial drug use could be achieved through improving the diagnosis of malaria. Although programs such as IMCI aim to improve clinical diagnosis through well-designed clinical algorithms, a large number of patients will continue to receive unnecessary antimalarial therapy, especially in areas of relatively low malaria risk (18). Basing treatment on the results of a diagnostic test, such as microscopy or a rapid antigen test, however, would result in the greatest reduction of unnecessary malaria treatments and decrease the probability that parasites are exposed to subtherapeutic blood levels of drug.

There are notable exceptions to this, however. Presumptive therapy with SP during pregnancy has been shown to be an operationally sustainable intervention that offers significant protection from low birth weight associated with placental malaria (62). There may be a role for presumptive treatment or even mass drug administration in response to an epidemic, although its cost-effectiveness has not been proven. Prophylaxis programmes, however, should be used only among populations where compliance is likely to be high and where a highly effective prophylactic drug can be used.

6.1.2 Improving the way drugs are used

Other disease control programmes, such as for TB, STDs, and HIV, have begun to rely heavily on directly observed therapy (DOT) as a way to ensure a high degree of compliance. While this has not yet received serious consideration for malaria, the use of drugs with single-dose regimens (SP, mefloquine) could potentially make DOT possible. The benefits of using single-dose DOT need to be weighed against the costs of using drugs with long half-lives.

Another approach that has not been widely adopted is the close follow-up and re-treatment, if necessary, of patients. The success of this approach is dependant on availability of reliable microscopy (to diagnose the illness initially as well as to confirm treatment failure), and either an infrastruc-

ture to locate patients in the community or a community willing to return on a given date, regardless of whether they feel ill or not. With this system, patients who fail initial treatment, for whatever reason, are identified quickly and re-treated until parasitologically cured, decreasing the potential for spread of resistant parasites (107).

6.1.3 Combination therapy

A strategy that has received much attention recently is the combination of antimalarial drugs, such as mefloquine, SP, or amodiaquine, with an artemisinin derivative (108). Artemisinin drugs are highly efficacious, rapidly active, and have action against a broader range of parasite developmental stages. This action apparently yields two notable results. First, artemisinin compounds, used in combination with a longer acting antimalarial, can rapidly reduce parasite densities to very low levels at a time when drug levels of the longer acting antimalarial drug are still maximal. This greatly reduces both the likelihood of parasites surviving initial treatment and the likelihood that parasites will be exposed to suboptimal levels of the longer acting drug (32). Second, the use of artemisinins has been shown to reduce gametocytogenesis by 8- to 18-fold (33). This reduces the likelihood that gametocytes carrying resistance genes are passed onwards and potentially may reduce malaria transmission rates. Use of combination therapy has been linked to slowing of the development of mefloquine resistance and reductions in overall malaria transmission rates in some parts of Thailand and has been recommended for widespread use in sub-Saharan Africa (108). This interpretation and the recommendation for rapid adoption of combination therapy in Africa, however, has been questioned (109, 110).

It should be noted that this argument contradicts a previously mentioned argument in that it promotes the use of a drug combination with grossly mismatched half-lives. Theoretically, in areas where malaria transmission rates are quite low, such as where this strategy has been most intensively studied in Thailand, this is of minimal concern (i.e. the likelihood of being bitten by an infective mosquito during the period when drug levels are suboptimal is very low). In areas where transmission rates are very high (for example, Africa where inoculation rates can be as high as five infective bites per night), this likelihood is very high. The relative contribution to development of resist-

ance of parasites surviving initial malaria treatment compared with new parasites being exposed to sub-optimal drug levels is unknown.

As second concern about combination therapy is the extent to which the components might be used for monotherapy outside official health services. Already, artemisinin compounds are available in the pharmacies and markets of Africa. As supply increases and the price drops, these drugs will be used increasingly for the treatment of fever and, because of the rapidity of action, they may in fact become the community's drug of choice. It is unlikely, in this scenario, that they would be used in combination with another drug, whether SP or mefloquine. Similarly, in Africa, SP is both widely available and inexpensive and may continue to be used alone. Any benefits of combination therapy in preventing development or intensification of resistance may be lost due to unofficial and incorrect use of the component drugs outside of official health services.

In the future, antimalarial therapy may be expanded by combining chemotherapy with vaccines (or other drugs) specifically designed to inhibit transmission of malaria. These "transmission-blocking" vaccines or drugs could reduce the potential for onward transmission of gametocytes carrying resistance genes, even if a relatively large number of parasites survive initial treatment. This could work through using drugs or vaccines with a high degree of specific antigametocytocidal activity (such as primaquine and related drugs), drugs that non-specifically reduce the likelihood of gametocytes developing (such as appears to be the case with the artemisinins), or drugs or vaccines that interfere with sexual reproduction and infection of the parasite within the mosquitos when taken up with a blood meal (although short acting, the combination of atovaquone and proguanil has this type of activity).

7. Conclusions and recommendations

Because of the realities of health care infrastructure and the influence of the private sector, approaches to malaria therapy, especially in sub-Saharan Africa, will probably favour increased access to drugs (and, therefore, loss of control over how they are used) over restricted access (and, therefore, more control over how they are used). If this proves to be true, while only minor advances against antimalarial drug resistance can be expected, short-term reductions in malaria morbidity and mortality may be achieved.

Long-term success of this strategy, however, will *depend* on a continuous supply of new and affordable drugs and on the development of effective and implementable control measures to reduce overall burden of disease. A more cautious approach would be to avoid placing too much faith in future scientific advances and technology and to invest in methods to improve the way people and antimalarial drugs interact in an environment of essentially uncontrolled use. The objective of this investment would be to prolong the useful life span of drugs enough to increase the likelihood that new drugs or other methods of malaria control will indeed be developed and implemented.

Significant advances against antimalarial drug resistance is probably unlikely without major change in health infrastructure leading to higher-quality services that are more readily available.

7.1 Priorities

- A. Investigate combination therapy:
1. Fast-track a chlorproguanil/dapsone/artesunate fixed dose formulation. From a theoretical basis, this would offer the best combination of overall efficacy, synergy between the antifolate-sulfa components, short half-life, reasonably well-matched pharmacokinetics, and probable cost. Because of growing use of and resistance to SP, an urgency exists to field this promising agent.
 2. Investigate effectiveness of combination therapy in terms of robustness of strategy in face of high levels of self-treatment and unofficial use of component drugs (or related compounds) as monotherapy and in various epidemiological contexts (especially high-transmission areas).
3. Investigate how a combination therapy strategy could be financed. This strategy, if proven cost-effective, will nonetheless be more expensive than current strategies. What mechanisms might be developed to assist countries in adopting this strategy?
- B. Invest significantly in identifying strategies to improve acceptance of and compliance with drug regimens, especially a combination therapy strategy, at all levels of official and unofficial health care systems, private sector, and community. Similarly, investigate to teach concepts of judicious use of antimicrobials (including antimalarial drugs) to health care providers.
- C. Investigate ways to improve effectiveness of drug regulatory systems and ability to control introduction of new antimalarials within endemic countries. This is required to avoid uncontrolled use of new antimalarials resulting in development of resistance before they are needed which could significantly compromise their efficacy when they are needed.
- D. Support new drug development. Investigate new approaches to drug delivery, such as time-released formulations or novel delivery systems that would allow use of short half-life drugs while optimizing compliance. Investigate drugs (or vaccines?) that have transmission-blocking effect that could be used in combination with drugs active against blood-stage parasites.
- E. Improve access to and use of definitive diagnosis-based treatment.
- F. Support more widespread use of insecticide-treated materials or other appropriate vector control strategies to reduce frequency of clinical illness (and therefore, treatment) as well as overall malaria transmission.

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