Antimalarial drug resistance in Africa: the calm before the storm?

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Antimalarial drug resistance, in particular resistance to *Plasmodium falciparum*, challenges the treatment and control of malaria. In this Review, we summarise evolving patterns of antimalarial drug resistance in Africa. Resistance to aminoquinolines and antifolates is long-standing, yet with greatly decreased use of chloroquine to treat malaria, the prevalence of resistance to chloroquine has decreased. Resistance to antifolates, which are used to prevent malaria in some settings, remains widespread. Resistance to artemisinin-based combination therapies, the standard treatments for malaria in Africa, has emerged in southeast Asia. At present, resistance to artemisinins or key partner drugs included in combination therapies does not appear to be a substantial problem in Africa. However, emergence of resistance to artemisinin-based combination therapies in Africa would probably have devastating consequences, and continued surveillance for the emergence of resistance on this continent is a high priority.

Lancet Infect Dis 2019; 19: e338–51

Published Online July 30, 2019 http://dx.doi.org/10.1016/S1473-3099(19)30261-0

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Antimalarial drug use in Africa

Malaria remains an overwhelming problem in Africa, where about 90% of global malaria morbidity and mortality occur.[1] In most of sub-Saharan Africa, the majority of cases are caused by *Plasmodium falciparum*, the most virulent human malaria parasite, and the parasite with the greatest likelihood of drug resistance. *Plasmodium vivax* causes large numbers of malaria cases in Ethiopia and Madagascar, and small numbers of cases elsewhere.[2] *Plasmodium ovale* and *Plasmodium malariae* cause fewer cases than *P falciparum* across the continent. In this Review, we focus on *P falciparum* because of its prominent role in Africa, and because information on resistance in the other species that cause malaria in Africa is very scarce. We aim to summarise the use of antimalarial drugs in Africa, how drug sensitivity has evolved over time, and current evidence regarding resistance to artemisinin-based combination therapies (ACTs) in Africa.

The key role for antimalarial drugs in Africa is for the treatment of uncomplicated malaria (table 1). Uncomplicated disease makes up the large majority of episodes of malaria, and the disease is seen principally in children.[1] Treatment is focused on *P falciparum* malaria, and must be directed against potentially drug-resistant organisms. Since the early 21st century, the treatments of choice for malaria in Africa have been ACTs (table 2).[3] Nearly all countries in sub-Saharan Africa recommend artemether-lumefantrine, artesunate-amodiaquine, or either of these regimens for the treatment of uncomplicated malaria.[1,4] Other ACTs relevant for Africa and recommended by WHO are dihydroartemisinin-piperaquine, artesunate-mefloquine, and artesunate-pyronaridine.[4] Other regimens not endorsed by WHO for Africa but available in some settings include artesunate-sulfadoxine-pyrimethamine, artemisinin-piperaquine, artemisinin-naphthoquine, and arterolane-piperaquine.

The standard treatment for severe malaria is intravenous artesunate, which showed improved efficacy and decreased mortality compared with quinine in two major randomised trials, one of which included over 5000 African children.[5,6] Rectal artesunate,[7] intramuscular artesunate,[8] and rectal artemether[9] can be administered in settings where intra-venous therapy is not possible. Intravenous quinine remains an alternative therapy for severe malaria.[10]

In addition to treating acute illness, antimalarial drugs are used to prevent malaria in Africa. Strategies recommended by WHO are intermittent preventive therapy, with monthly sulfadoxine-pyrimethamine to prevent malaria during pregnancy,[11,12] and seasonal malaria chemoprevention, with monthly amodiaquine-sulfadoxine-pyrimethamine to decrease childhood malaria incidence during the rainy season in parts of the Sahel subregion with highly seasonal malaria transmission.[13] Additional strategies, including intermittent preventive therapy in other populations,[14] mass drug administration,[15,16] and reactive case detection and treatment,[17] are being studied. Chemoprophylaxis for malaria is a routine practice for travellers to Africa from regions not endemic for malaria, most commonly with atovaquone-proguanil, mefloquine, or doxycycline.[18] With changing epidemiology, this practice might increasingly be appropriate for African people travelling from low to high transmission areas on the continent.

Evolving resistance to antimalarial drugs in Africa

The use of drugs for the treatment or prevention of malaria in Africa is threatened by resistance. Resistance to older drugs, notably chloroquine and related aminoquinolines and antifolates, has been prevalent for decades. Newer ACTs appear to remain highly effective, but ACT resistance has emerged in southeast Asia.[19] It is noteworthy that resistance to many antimalarials, including chloroquine, mefloquine, quinine, and artemisinins, emerged independently outside of Africa. Resistance selection might be less likely in Africa compared with other areas because of the high level of immunity in African populations, the high level of complexity of African infections, and other factors, which limit the emergence of relatively unfit resistant strains.[20] Nonetheless, resistance to multiple classes of

	Use	Resistance polymorphisms	Situation in Africa
4-aminoquinolines			
Chloroquine	Treatment of non-*Plasmodium falciparum* malaria	*Pfcrt* 76Thr primary mediator; *pfmdr1* 86Tyr and 1246Tyr; other SNPs in these two genes contribute, mainly outside Africa	Resistance, mediated primarily by *pfcrt* 76Thr, has been widespread, but reversion to wild-type (sensitive) parasites is ongoing in many areas
Amodiaquine	Treatment in combination with artesunate	Impacted by same mutations as chloroquine, but active against resistant parasites	Cross-resistance with chloroquine, but artesunate-amodiaquine highly efficacious
Bis-quinoline			
Piperaquine	Treatment in combination with DHA	Increased plasmepsin-2 copy number; *pfcrt* SNPs	Highly effective in combination with DHA; resistance polymorphisms seen in southeast Asia are uncommon in Africa
Arylamino alcohols			
Quinine	Treatment, including severe malaria	SNPs in *pfmdr1*, *pfmdr6*, *pfcrt*, *pfmrp1*, and *pfnhe1* might be associated with resistance	Parasites generally susceptible
Mefloquine	Treatment or prophylaxis as monotherapy or in combination with artesunate	Increased *pfmdr1* copy number	Highly effective in combination with artesunate
Lumefantrine	Treatment in combination with artemether	Resistance not documented; decreased sensitivity associated with *pfcrt* and *pfmdr1* polymorphisms	Highly effective in combination with artemether
Mannich base			
Pyronaridine	Treatment in combination with artesunate	*pfcrt* 76Thr mutation associated with decreased ex vivo sensitivity	Highly effective in combination with artesunate
Artemisinins			
DHA	Treatment in combination with piperaquine	K13PD mutations	Highly effective as ACT component
Artemether	Treatment in combination with lumefantrine	K13PD mutations	Highly effective as ACT component
Artesunate	Treatment in combination with amodiaquine, mefloquine, or pyronaridine; severe malaria (intravenous)	K13PD mutations	Highly effective as ACT component
Quinone			
Atovaquone	Treatment and prophylaxis, both in combination with proguanil	Mutations in cytochrome b, particularly 268Ser, 268Cys, 268Asn	Resistance has been reported, but uncommon
Folate antagonists			
Pyrimethamine	Treatment in combination with sulfadoxine	Step-wise resistance with acquisition of *pfdhfr* mutations (108Asn, 51Ile, 59Arg, and 164Leu)	Widespread resistance
Proguanil	Treatment and prophylaxis, both in combination with atovaquone	Step-wise resistance with acquisition of *pfdhfr* mutations (108Asn, 51Ile, 59Arg, and 164Leu)	Widespread resistance
Sulfonamide folate antagonist			
Sulfadoxine	Treatment in combination with pyrimethamine	Step-wise resistance with acquisition of *pfdhps* mutations (primarily 437Gly, 540Glu, 581Gly)	Widespread resistance
Tetracycline antibiotic			
Doxycycline	Treatment in combination with quinine; prophylaxis	SNPs in *pfmdt* and *pftetQ*	Unclear if clinically relevant resistance is present
8-aminoquinolines			
Primaquine	Elimination of dormant stages *Plasmodium vivax* and *Plasmodium ovale*; elimination of gametocytes; prophylaxis	Resistance not documented	Little used in Africa; single-dose therapy being studied to control transmission
Tafenoquine	Elimination of dormant stages *P vivax* and *P ovale*; elimination of gametocytes; prophylaxis	Resistance not documented	Newly approved

SNP=single nucleotide polymorphism. DHA=dihydroartemisinin. ACT=artemisinin-based combination therapies.

Table 1: **Major antimalarial drugs and their resistance status in Africa**

antimalarial drugs has spread to Africa, and as transmission decreases in some areas, the likelihood of emergence of resistance might increase. Drug sensitivity in Africa is monitored on the basis of standardised clinical, parasitological, and molecular criteria (table 3). Next, we summarise resistance patterns and mechanisms of resistance for key antimalarial drugs in Africa.

	Use in Africa	Resistance situation worldwide	Situation in Africa
Artemether-lumefantrine	Widely used to treat uncomplicated malaria	Resistance not documented, but efficacy inferior to that of other ACTs in some settings	Excellent efficacy
Artesunate-amodiaquine	Widely used to treat uncomplicated malaria	Little used outside Africa	Excellent efficacy
DHA-piperaquine	Alternative for uncomplicated malaria	Resistance prevalent in Cambodia	Excellent efficacy
Artesunate-mefloquine	Little used	Resistance prevalent in parts of Thailand	Little used, but excellent efficacy
Artesunate-pyronaridine	Little used	Resistance not documented, but efficacy inferior to that of other ACTs in some settings	Little used, but excellent efficacy
Artesunate-sulfadoxine-pyrimethamine	Little used	Widespread resistance to sulfadoxine-pyrimethamine	Not tested; widespread resistance to sulfadoxine-pyrimethamine
Artemisinin-piperaquine*	Little used	Resistance presumably as for DHA-piperaquine	Little studied
Artemisinin-naphthoquine*	Little used	Resistance not documented	Little studied
Arterolane-piperaquine*	Little used	Resistance presumably as for DHA-piperaquine	Little studied
Sulfadoxine-pyrimethamine	Standard for IPTp and in combination with amodiaquine for SMC	Widespread resistance	Widespread resistance
Atovaquone-proguanil	Little used for treatment; prophylaxis in non-African travellers	Generally efficacious as therapy and prophylaxis, but resistant parasites seen uncommonly	Little studied; little used

ACT=artemisinin-based combination therapies. DHA=dihydroartemisinin. IPTp=intermittent preventive therapy in pregnancy. SMC=seasonal malaria chemoprevention. *Not recommended by WHO.

Table 2: **Antimalarial drug combinations available in Africa**

Quinine

Cinchona bark was used for the treatment of malaria beginning in the 1600s, and quinine has been a standard therapy for about 200 years.[10] Resistance to quinine was reportedly first noted in 1910, although it is difficult to assess reports that predate modern methods.[10] More recently, decreased quinine effectiveness has been reported in Asia and South America, but it appears to be uncommon in Africa.[10] Quinine is efficacious for the treatment of uncomplicated malaria, although compliance with a 7 day regimen is challenging, leading to suboptimal efficacy.[21] Although it is ideally replaced by artesunate, quinine also remains a reliable therapy for severe malaria in Africa. Mechanisms of resistance to quinine are uncertain, but polymorphisms in *pfmdr1*,[22–25] *pfcrt*,[23,24] *pfnhe1*,[23,24] *pfmrp1*,[26] and *pfmdr6*[27] might contribute.

	Advantages	Disadvantages
Clinical trial	True measure of treatment efficacy	Efficacy affected by factors independent of drug resistance (eg, immunity and absorption); drugs generally studied as combinations
Determination of ex-vivo drug sensitivity	Direct measure of drug sensitivity of parasites	Requires complex laboratory setting; expensive, laborious, prone to laboratory-to-laboratory variation
Assessment of molecular markers of resistance	Relatively simple assays; can use filter paper blood spot specimens	Associations between known polymorphisms and resistance phenotypes are incomplete

Table 3: **Principal methods for characterising antimalarial drug resistance**

Aminoquinolines

Chloroquine became the standard therapy for malaria in the 1940s. The drug offered a major advance, due to rapid action, simplified dosing, improved tolerability, and decreased toxicity compared with quinine. However, resistance to chloroquine was reported in the late 1950s in both southeast Asia and South America.[28] Chloroquine resistance spread slowly and was first identified in east Africa in 1979, before spreading across the continent.[28] Despite increasing resistance, chloroquine remained the drug of choice to treat uncomplicated *P falciparum* malaria in most African countries into the 21st century. In retrospect, continued use of chloroquine despite widespread resistance likely contributed to the deaths of millions of African children.

Chloroquine resistance is strongly associated with a single nucleotide polymorphism, resulting in a Lys76Thr change in the *pfcrt* gene, which encodes the chloroquine resistance transporter.[29,30] This protein is located on the parasite's digestive vacuole membrane and appears to mediate efflux of chloroquine from this organelle.[30] The Lys76Thr mutation mediates resistance in vitro, but in field isolates, it is associated with various other mutations that might modify resistance phenotypes and appear to be needed to maintain fitness of resistant parasites.[31] Other patterns of *pfcrt* mutations have been associated with varied sensitivity to chloroquine and other amino-quinolines outside of Africa,[32–35] but in Africa, the *pfcrt* Lys76Thr mutation appears to be a reliable marker for chloroquine resistance.[36,37] Mutations in another putative

transporter, the multidrug resistance protein encoded by *pfmdr1*, are also associated with sensitivity to a number of antimalarials.[38,39] The Asn86Tyr, Tyr184Phe, and Asp1246Tyr mutations are common in Africa, and the Asn86Tyr mutation and particular haplotypes are associated with decreased sensitivity to chloroquine.[36,40] Other polymorphisms in *pfmdr1*, including gene amplification, have been associated with altered drug sensitivity in other regions.[41,42] However, these polymorphisms have not commonly been reported in African parasites,[43–45] and have generally been detected at low prevalence,[46–49] with a few exceptions.[50–52]

By the 1990s, resistance to chloroquine was widespread in east and southern Africa, with prevalence of the *pfcrt* Lys76Thr mutation near 100% (for comprehensive information on *P falciparum* resistance-mediating polymorphisms see the WorldWide Antimalarial Resistance Network[53]). This mutation was also widespread in other areas of Africa, although it did not reach fixation in some areas. Changes in treatment regimens have been followed by marked changes in circulating parasites, with reversion to chloroquine sensitivity (figure 1). This reversion was first noted in Malawi, which changed its first-line regimen from chloroquine to sulfadoxine-pyrimethamine in 1993, well before changes in other African countries. Over the next decade, *P falciparum* in Malawi showed reversion to wild type, with loss of the *pfcrt* Lys76Thr mutation[54] and gain of sensitivity to chloroquine in ex-vivo assays.[54] These changes were associated with excellent therapeutic efficacy of chloroquine in the treatment of uncomplicated malaria.[55] With replacement of chloroquine by ACTs across Africa early in the 21st century, reversion to parasites with wild-type *pfcrt* and *pfmdr1* sequences has also been seen (figure 1). Marked changes in polymorphism prevalence over recent years mean that in parts of east Africa most parasites now have chloroquine sensitive genotypes[57–58] and ex-vivo phenotypes.[36,37] These changes are probably selected both by decreased chloroquine pressure and by the selective pressure of lumefantrine, a component of the widely used ACT artemether-lumefantrine, which selects in the opposite direction to aminoquinolines at the key *pfcrt* Lys76Thr and *pfmdr1* Asn86Tyr loci.

Sensitivity to amodiaquine, an aminoquinoline that is related to chloroquine and is a component of the ACT artesunate-amodiaquine, appears to be mediated by the same mutations as sensitivity to chloroquine. However, *pfmdr1* mutations might be more important for amodiaquine sensitivity than for chloroquine sensitivity, and amodiaquine remains active against many relatively resistant parasites because its potency is greater than that of chloroquine. Use of artesunate-amodiaquine selected strongly for the *pfcrt* Lys76Thr, *pfmdr1* Asn86Tyr, and *pfmdr1* Asp1246Tyr mutations in parasites that emerged soon after therapy,[59–62] and for parasites with decreased ex-vivo sensitivity to desethylamodiaquine (the active metabolite of amodiaquine),[63] supporting roles for these polymorphisms in mediating amodiaquine sensitivity. In addition, the South American *pfcrt* SVMNT (representing the sequence at amino acids 72–76) allele has been associated with greater resistance to amodiaquine compared with the African and southeast Asian CVIET allele.[64] The SVMNT allele has been reported at low prevalence in parts of Africa.[65–67]

The bisaminoquinoline piperaquine appears to remain active against chloroquine resistant parasites, and resistance to piperaquine has not been evident in Africa, although in Uganda,[45,68–70] but not in Burkina Faso,[71,72] treatment with dihydroartemisinin-piperaquine selected for the same *pfcrt* and *pfmdr1* mutations as artesunate-amodiaquine in infections that emerged after therapy. Importantly, decreased activity of piperaquine has been reported in Cambodia,[35,73–76] where it has been associated with increased copy number of plasmepsin aspartic protease genes,[77,78] a single nucleotide polymorphism in an exonuclease gene,[77] and novel mutations in *pfcrt*.[33,34] Recent analyses of African isolates have shown low (generally <10%) prevalence[79–81] of increased plasmepsin copy number (with the exception of one report)[82] and no convincing evidence that this or other polymorphisms are mediating piperaquine resistance in Africa.[36]

Polymorphisms in other proteins have been associated with altered drug sensitivity in a few studies with African isolates. Single nucleotide polymorphisms in the multidrug resistance protein encoded by *pfmrp1* were selected by artemether-lumefantrine in isolates from several countries in Africa,[83] but not by artemether-lumefantrine or dihydroartemisinin-piperaquine in Uganda.[45] Variations in microsatellite repeats in *pfnhe1*, which encodes a putative sodium–hydrogen exchanger, have been seen in African isolates, and associated with altered sensitivity to quinine in some, but not other, studies.[84–88] Analysis of *P falciparum* genotypes 2–3 days after therapy with artemether-lumefantrine or dihydroartemisinin-piperaquine in Kenya showed increased prevalence of some polymorphisms in *pfap2-mu* and *pfubp1*, suggesting selection of polymorphisms that mediate altered drug response.[89]

Lumefantrine, mefloquine, and pyronaridine

Lumefantine, mefloquine, and pyronaridine are important ACT partner drugs that appear to be consistently highly effective against *P falciparum* in Africa. Activity of lumefantrine, a component of the most widely used ACT, artemether-lumefantrine, is well studied. Lumefantine has never been available as a monotherapy, and it generally shows excellent antimalarial activity. Some studies have shown worrying decreased activity of lumefantrine (50% inhibitory concentration [IC_{50}] >50–100 nM), but these results could be due to inadequate solubility of lumefantrine, or other factors, leading to spurious results.[37,90,91] With careful attention to drug solubility, IC_{50} values were consistently less than 25 nM at two sites in Uganda from 2006 to 2016,[36,68,92] with values

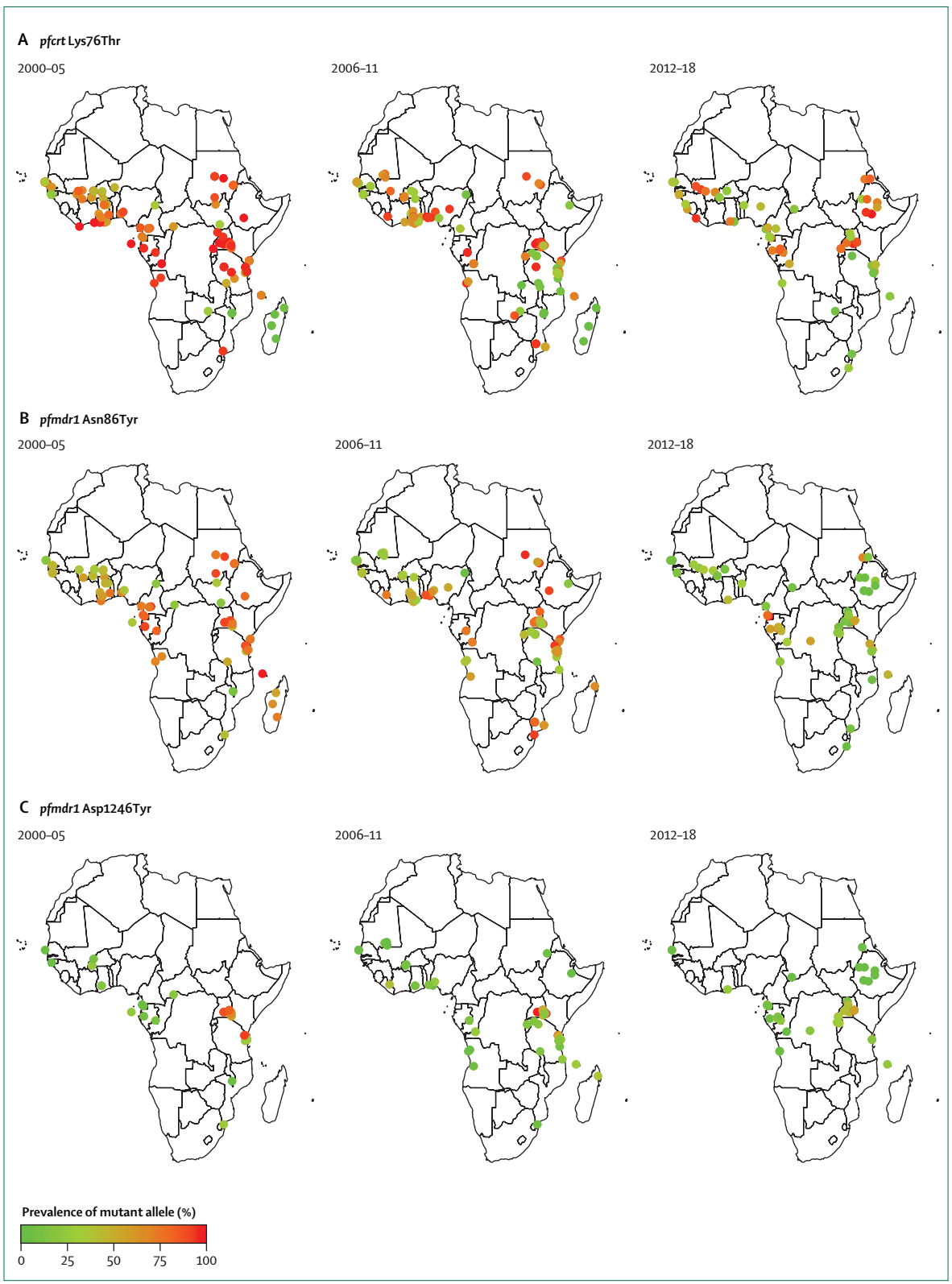

Figure 1: **Distribution of *Plasmodium falciparum* transporter polymorphisms (*pfcrt* Lys76Thr [A], *pfmdr1* Asn86Tyr [B], and *pfmdr1* Asp1246Tyr [C]) associated with altered drug sensitivity across Africa over time**

Circles indicate sites of studies that evaluated at least 15 isolates that are included in the WorldWide Antimalarial Resistance Network Partner Drug Molecular Surveyor. The prevalence of mutations (considering the 3D7 strain wild type and including mixed genotypes) is indicated by the colour scale.

similar to those reported in 1997 and 2003 in Cameroon.[93] However, use of artemether-lumefantrine is accompanied by selection of wild-type sequences in putative drug transporters—namely *pfcrt* Lys76Thr and *pfmdr1* Asn86Tyr and Asp1246Tyr in parasites that emerge soon after therapy.[60,61,71,94–97] This wild-type sequence selection is opposite to the selective pressures of artesunate-amodiaquine and dihydroartemisinin-piperaquine, which select for mutant sequences at these alleles, as previously mentioned in this Review. Selection of these wild-type sequences by artemether-lumefantrine was accompanied by modest decreases in lumefantrine activity,[36] and, although the clinical efficacy of artemether-lumefantrine in Africa has been excellent, a pooled analysis showed that the *pfmdr1* Asn86 wild-type sequence was a significant risk factor for recrudescence after treatment with artemether-lumefantrine.[98] Mefloquine has been little used in Africa; it appears to have the same selective pressure as lumefantrine.[99] In Asia, both mefloquine and lumefantrine also select for increased *pfmdr1* copy number, associated with decreased activity against *P falciparum*,[41,42] but in isolates from Africa, increased copy number is reported infrequently, even after use of artemether-lumefantrine.[43–45] Pyronaridine has shown potent activity against African strains of *P falciparum*;[100–102] cross-resistance with aminoquinolines has generally not been seen, although association between the *pfcrt* Lys76Thr mutation and decreased ex-vivo sensitivity has been reported.[103]

See **Online** for appendix

Artemisinins

Resistance to artemisinins, manifested as delayed clearance of parasites after therapy with artemisinins, is widespread in the Greater Mekong subregion of southeast Asia.[104–106] The clinical phenotype of artemisinin resistance is associated with several kelch protein propeller domain (K13PD) mutations[107] and delayed in-vitro clearance in the ring survival assay.[108,109] Although the delayed clearance artemisinin-resistance phenotype alone does not lead to frequent failures of combination therapy, loss of activity of the partner drugs mefloquine[110,111] and piperaquine[76–78] has led to frequent failures of ACTs for the treatment of *P falciparum* malaria in parts of southeast Asia.

Experience from southeast Asia provides valuable metrics to assess artemisinin resistance in Africa (table 4). Evidence suggests continued excellent activity of artemisinins against African *P falciparum*. Delayed clearance after therapy has been very uncommon in African trials, and a meta-analysis involving almost 30 000 treatments across Africa showed less than 1% persistent parasitaemia on day 3 after initiation of therapy.[112] The standard ring survival assay, an in-vitro marker for delayed clearance, showed that African isolates rarely had parasitaemia of more than 10% 72 h after a 6 h incubation with 700 nM dihydroartemisinin.[109] Using this assay, parasitaemia of more than 10% at 72 h was found in none of 12 isolates that were collected in Mali in 2010 and 2011,[113] none of 43 in Uganda in 2014,[114] none of 64 in Cameroon in 2015,[115] none of 32 in Uganda in 2016,[36] and four of 194 in Uganda from 2014 to 2016.[116]

Multiple K13PD polymorphisms have been associated with artemisinin resistance in southeast Asia;[107] a meta-analysis[117] confirmed 20 different mutations as associated with the resistant phenotype.[118] Extensive evaluations of African isolates have shown mutations for at least 154 different K13PD amino acids (defining the propeller domain as between residues 416 and 692), with usually only one mutation per isolate, and overall prevalence of mutant isolates generally less than 5% (appendix).[119–121] The Ala578Ser K13PD mutation is the most widespread in Africa, with identification in at least 22 countries, and haplotype analysis suggesting multiple independent emergences.[121] Introduction of the Ala578Ser mutation into *P falciparum* did not lead to altered artemisinin sensitivity,[121] consistent with this mutation not mediating resistance. Most of the K13PD mutations identified in African isolates are not among those definitively associated with delayed clearance of *P falciparum* in southeast Asia, and sequence analysis suggests little local selection of K13PD mutations.[122] A study from northern Uganda showed enhanced in-vitro ring survival for four of 194 isolates, and one of these four isolates contained a K13PD mutation.[116] This mutation, Ala675Val, has been associated with delayed clearance in southeast Asia.[117] The Ala675Val mutation was seen in 5% of isolates collected from four sites in northern Uganda in 2017;[123] with only a single isolate with this genotype and enhanced in-vitro ring survival, its clinical relevance is uncertain.

Antifolates

Antifolates, including pyrimethamine, alone or in combination with dapsone or sulfadoxine, have been widely used to treat malaria, and sulfadoxine-pyrimethamine replaced chloroquine to treat malaria in some African countries. Establishment of sulfadoxine-pyrimethamine as the first-line antimalarial therapy in Malawi was associated with rapid selection of parasites with *pfdhfr* mutations that mediate decreased drug sensitivity.[54] Resistance to antifolates, mediated by mutations in the target *pfdhfr* and *pfdhps* genes, and selected in a step-wise fashion or spread in selective sweeps, is widespread.[124–126] In much of Africa, five mutations (*pfdhfr* Asn51Ile, Cys59Arg, Ser108Thr or Ser108Asn; *pfdhps* Ala437Gly, Lys540Glu) are very common, and mediate an intermediate level of resistance that seriously compromises the activity of sulfadoxine-pyrimethamine. One of these mutations, *pfdhps* Lys540Glu, is mostly absent in much of west and central Africa,[127] with a distinct boundary for high prevalence shown in the Democratic Republic of Congo (figure 2).[128] Improved activity of sulfadoxine-pyrimethamine in west Africa because of absence of this mutation is probably the reason for continued good efficacy of amodiaquine plus sulfadoxine-pyrimethamine,[61,129] and this regimen is now recommended

	Outcomes predicting resistant phenotype	Situation in southeast Asia	Situation in Africa
Clinical trial	Delayed parasite clearance; failures of treatment with ACTs	Delayed clearance and failures of some ACTs in Greater Mekong subregion	Delayed clearance and failures of ACTs uncommon
Sequencing of K13	Various propeller domain polymorphisms	Many K13PD polymorphisms seen; Cys580Tyr dominates in many areas	Many polymorphisms seen, but mostly not those associated with delayed clearance in southeast Asia
Ring survival assay	Survival greater than that in control strains at 48 h	Abnormal ring survival assay common, correlating with delayed clearance and K13PD polymorphisms	Abnormal ring survival assay uncommon

ACT=artemisinin-based combination therapies.

Table 4: **Principal methods for identifying the artemisinin resistance (delayed clearance) phenotype**

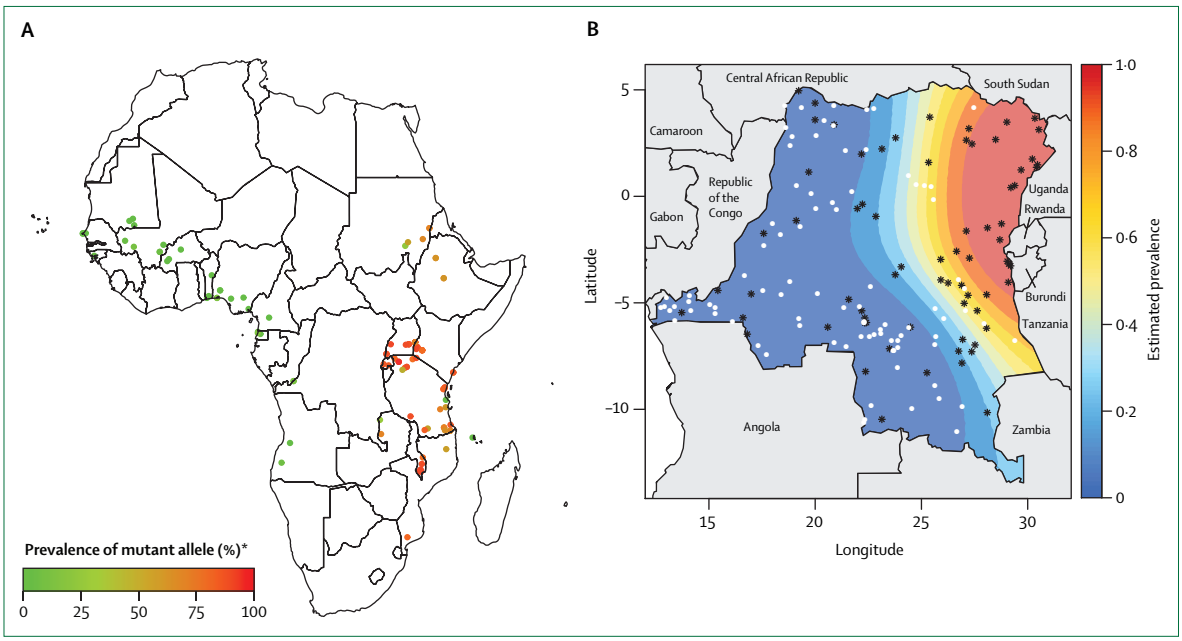

Figure 2: **Distribution of *pfdhps* Lys540Glu polymorphisms across Africa 2003–15 (A) and estimated prevalences of *pfdhps* Lys540Glu mutations in the Democratic Republic of the Congo 2013–14 (B)**

(A) Circles indicate sites of studies, chosen and described as in figure 1. *Mixed genotypes included. (B) White circles indicate sites where only wild-type alleles were found and black stars indicate sites where at least one mutation was found. Contour lines indicate 10% prevalence levels (adapted from Ayedemir et al, 2018).[126]

by WHO, with proven efficacy, for seasonal malaria chemoprevention in regions of the Sahel sub-region with highly seasonal malaria.[130–132] Sulfadoxine-pyrimethamine also remains the standard-of-care for intermittent preventive therapy in pregnant women in all regions of Africa that are endemic to malaria, although its efficacy is limited by resistance.[11] Of concern, additional mutations, notably *pfdhfr* Ile164Leu and *pfdhps* Ala581Gly, are now quite common in parts of east Africa.[57,123,133–135] These mutations will probably further compromise the antimalarial activity of sulfadoxine-pyrimethamine. Indeed, the *pfdhps* Ala581Gly mutation has been associated with decreased preventive efficacy of sulfadoxine-pyrimethamine in pregnant women.[136] On the basis of these results, WHO has recommended use of sulfadoxine-pyrimethamine for intermittent preventive therapy in children only when the *pfdhps* Lys540Glu mutation is absent. But, in practice, sulfadoxine-pyrimethamine is not used to prevent malaria in children in Africa, except in combination with amodiaquine for seasonal malaria chemoprevention, as described previously. Therefore, a pressing need is a regimen to replace sulfadoxine-pyrimethamine for intermittent preventive therapy in regions where sulfadoxine-pyrimethamine efficacy is inadequate.

Primaquine and tafenoquine

Primaquine has broad antimalarial activity, but is principally used to eliminate dormant liver stages of *P vivax* and *P ovale* (a process referred to as radical cure), and is increasingly used in *P falciparum* treatment regimens to eliminate sexual stages of the parasite and therefore prevent transmission to mosquitoes.[137] Inclusion of primaquine in antimalarial regimens has not been common in Africa because of potential haemolysis in individuals who are glucose-6-phosphate dehydrogenase

deficient, although low-dose regimens appear to be safe.[138] Primaquine activity against *P vivax* varies in other regions of the world, but there is no definitive evidence for resistance. Tafenoquine, a related 8-aminoquinoline with prolonged exposure, was approved by the US Food and Drug Administration in 2018 for adults, for both the single-dose radical cure of *P vivax* infection and for chemoprophylaxis against malaria.[139–141] Information on resistance to tafenoquine is not available.

Antibiotics

Some antibiotics, notably tetracyclines, clindamycin, and macrolides, offer slow antimalarial activity, and these are included in some combination treatment regimens, such as quinine-doxycycline and, for children, quinine-clindamycin. Daily doxycycline is also used for chemoprophylaxis against *P falciparum* malaria. In one study,[142] increased copy number and sequence polymorphisms in the *pfmdt* and *pftetQ* genes were associated with decreased doxycycline sensitivity in African isolates, but it is unclear if clinically relevant decreases in doxycycline sensitivity are present in African parasites.

Atovaquone-proguanil

Atovaquone-proguanil is a standard therapy for malaria and is the most widely used regimen for the prevention of malaria in travellers from developed countries to Africa. However, the regimen is little used in Africa because of the high cost and the likelihood of selection of resistance with widespread use. Resistance is selected readily to both atovaquone, an inhibitor of cytochrome b (resistance mediated by *pfcytb* mutations), and proguanil, an inhibitor of *pfdhfr* (resistance mediated by the mutations noted for antifolate resistance). However, the synergistic combination of these two drugs appears to remain active against parasites with resistance to either component, and its utility for more widespread treatment or prevention of malaria in Africa is uncertain. Failures of atovaquone-proguanil for treatment of *P falciparum* malaria acquired in Africa have been associated with *P falciparum pfcytb* mutations, most commonly Tyr268Ser.[143–146] Of note, parasites resistant to atovaquone with mutations in *pfcytb* were unable to infect anopheline mosquitoes in laboratory studies, suggesting that these resistant parasites will not be transmitted in the field.[147]

Clinical antimalarial efficacy of ACTs in Africa

A crucial advance in malaria control was the widespread adoption of ACTs as the standard-of-care to treat uncomplicated *P falciparum* malaria in Africa early in the 21st century. In general, artemether-lumefantrine is the first-line therapy in east and southern Africa, either artemether-lumefantrine or artesunate-amodiaquine is first-line in central and west Africa, and dihydroartemisinin-piperaquine is an alternative regimen in some countries (table 2). In many countries, a range of ACTs, including WHO-recommended regimens and some others, are available in the public sector.

Numerous trials of the three leading ACTs have been done across Africa. Early studies[148,149] showed excellent antimalarial efficacy, significantly superior to that of non-ACT regimens, for artemether-lumefantrine and artesunate-amodiaquine. Subsequent trials[150,151] showed similar excellent efficacy for dihydroartemisinin-piperaquine, and more recent trials[62,152,153] have continued to show outstanding efficacy for artemether-lumefantrine, artesunate-amodiaquine, and dihydroartemisin-piperaquine, with genotype-corrected efficacies (considering only true recrudescences as treatment failures) generally over 95%. With uncorrected efficacies (considering all recurrences after therapy), the longer-acting ACT, dihydro-artemisinin-piperaquine, shows decreased recurrences during the 42 days after therapy compared with other ACTs.[152,153] Delayed clearance after therapy is very uncommon in Africa.[112]

Are there early signs of decreasing ACT efficacy in Africa?

As noted, an abundance of evidence, including clinical, parasitological, and molecular data, suggests that artemisinin resistance is not yet prevalent in Africa and that ACTs continue to offer outstanding antimalarial effectiveness. However, some reports suggest early signs of loss of ACT efficacy. Such reports should be interpreted with caution, because studies of drug resistance are necessarily complex, and there is an inherent bias toward the publication of newsworthy accounts. But because the spread of ACT resistance to Africa will have profound consequences, it is important to carefully assess all available data.

Case reports have described failed therapy with ACTs in individuals who have returned to other countries after acquiring malaria in Africa. A Vietnamese man presented in 2013 in Vietnam with severe *P falciparum* malaria 11 days after returning from Angola.[154] The patient remained parasitaemic despite 6 days of therapy with intravenous artesunate and then 3 days of therapy with dihydroartemisinin-piperaquine; parasitaemia resolved only after another 6 days of therapy with quinine-doxycycline. The K13PD sequence of the infecting isolate was not obtained. A Chinese man presented in 2013 with *P falciparum* malaria in China about 6 weeks after returning from Equatorial Guinea, where he was treated for malaria six times over 20 months.[155] The patient was treated with directly observed dihydroartemisinin-piperaquine, and had persistence of parasitaemia on day 3 after initiation, but clearance by day 7; the infecting parasite had a K13 Met579Ile mutation. Four patients in Sweden treated with artemether-lumefantrine in 2012–15,[156] four patients in the UK treated with artemether-lumefantrine in 2015–16,[157] and two patients in Italy treated with dihydroartemisinin-piperaquine in 2014–16;[158,159] all with uncomplicated *P falciparum* malaria acquired in Africa,

experienced late treatment failures. Most of these patients were non-immune European individuals, compliance with treatment regimens was uncertain, and all infecting parasites had wild-type K13PD sequences. Considering these case reports, treatment failures are of concern, but evidence that true artemisinin-resistant infections were acquired in Africa is not convincing.

A study[160] from the coast of Kenya in 2005–08 showed decreased rates of parasite clearance and increased recrudescence over time after treatment with artemether-lumefantrine or dihydroartemisinin-piperaquine. These results might suggest decreases in drug efficacy, but a more likely explanation is a decrease in antimalarial immunity in a region with decreasing malaria transmission intensity over this time frame.[160] In Uganda, among 78 children diagnosed with severe malaria, three had isolates with the Ala578Ser K13PD mutation, and parasite clearance was delayed in these children compared with the full cohort.[161] Reports from Angola (that used various statistical methodologies to assign outcomes) have suggested the possibility of decreased antimalarial efficacy of artemether-lumefantrine. In a 2013 trial[162] comparing artemether-lumefantrine and dihydroartemisinin-piperaquine for uncomplicated malaria, genotype-corrected recrudescence at 28 days was reported in 12% of children treated with artemether-lumefantrine in Zaire Province, compared with 3% in Uíge Province, and 0% of children treated with dihydroartemisinin-piperaquine in either province. In a similar trial[163] done in 2015, recrudescence at 28 days after treatment with artemether-lumefantrine was seen in 14% of children from Zaire Province, and 4% of children from Benguela Province. In these two Angolan studies, no isolates associated with treatment failure had K13PD mutations. In another trial,[48] done earlier (2011–13) in Luanda, Angola, recrudescence at 28 days was seen in 9% of children treated with artemether-lumefantrine. The studies from Angola reported concerning levels of recrudescence, but the most worrying results, from Zaire Province, were not accompanied by the K13PD markers for the artemisinin resistance phenotype, genotype correction in clinical trials is inexact, and it is unclear if identified decreases in artemether-lumefantrine efficacy are reasons for alarm. Reassuringly, a more recent trial[164] from Angola, done in 2017 in Zaire Province and other provinces, showed genotype-corrected efficacies of more than 95% in all studied provinces for artemether-lumefantrine, artesunateamodiaquine, and dihydroartemisinin-piperaquine.

Future use of ACTs and other drugs in Africa

Evidence suggests that African malaria parasites are increasingly sensitive to our current antimalarial armamentarium. Resistance to antifolates remains common, but parasites are increasingly sensitive to chloroquine and related aminoquinolines. The gain in activity of aminoquinolines has been accompanied by a small decrease in activity of lumefantrine[36] and a very modest decrease in efficacy of artemether-lumefantrine,[98] but overall efficacy of artemether-lumefantrine remains excellent. Activity of lumefantrine and mefloquine might be further compromised by parasites with increased *pfmdr1* copy number, but this has not commonly been seen in Africa. Newer markers of resistance, notably specific K13PD mutations associated with artemisinin resistance and increased plasmepsin gene copy number associated with piperaquine resistance, have been uncommon in surveillance studies in Africa, with identified polymorphisms not clearly associated with changes in drug sensitivity. In summary, although there should still be concern about impending spread or emergence of ACT resistance in Africa, African countries are currently in a calm before the storm, benefiting from excellent activity of multiple available antimalarial regimens. How can this situation be best taken advantage of?

One option is to restrict the use of antimalarial drugs as much as possible, to minimise selective pressure for drug resistance. With this scenario, physicians should continue to use artemether-lumefantrine and artesunate-amodiaquine to treat uncomplicated malaria, but they might avoid use of other ACTs for treatment, and not use ACTs for other indications, such as chemoprevention or mass drug administration, to avoid potential selection of resistant parasites. However, although the eventual spread of resistance is likely, this spread is probably more likely from importation of resistant Asian parasites than from de-novo selection of resistance, consistent with the spread of resistance to other antimalarials in Africa.[165] The risk of facilitating resistance selection by use of the most effective drugs could be small, compared with large potential benefits from using the best drugs. Therefore, an appealing alternative option, albeit with some potential to increase resistance selection, is to take advantage of the present activity of many antimalarials to scale up drug-based malaria control and elimination efforts. Potential strategies include: more widespread use of intermittent preventive therapy in children; chemoprevention in other high-risk groups, such as travellers from low to high transmission regions of Africa; mass drug administration; reactive case detection, with treatment of infected contacts of people with malaria in low transmission areas; and targeted elimination, treating all neighbours of index cases in low transmission areas. For many of the prevention strategies, the most promising available regimen is dihydroartemisinin-piperaquine, which, because of the prolonged kinetics of piperaquine, has shown excellent protective efficacy for about a month after administration in infants,[166] schoolchildren,[14] and pregnant women.[167,168] Combining highly effective preventive therapy with leading mosquito control measures is likely to offer major benefits, even in high-transmission areas. Subsequently, with development of new antimalarial agents, including those with features particularly appropriate for chemoprevention,[169] new artemisinin-free

Search strategy and selection criteria

We searched PubMed for the terms "antimalarial resistance", "malaria", "*Plasmodium*", "Africa", "pfmdr1", "pfcrt", "kelch", or "K13" to identify papers published between Jan 1, 2000 and Dec 31, 2018 on antimalarial drug sensitivity and resistance in Africa. Additionally, we reviewed relevant articles cited in those references and included them as primary sources when appropriate. The number of relevant studies was too large for all to be cited here, but all were considered, with comprehensive clinical trials, evaluations of ex-vivo drug sensitivity, and surveys of molecular resistance markers prioritised for citation. We also used the WorldWide Antimalarial Resistance Network (WWARN) Molecular Surveyor, to identify relevant studies of resistance markers.

For the **WWARN Molecular Surveyor** see http://www.wwarn.org/tracking-resistance

regimens for treatment and chemoprevention can be rolled out. In any event, continued surveillance for the spread of antimalarial drug resistance in Africa will remain a high priority.

Contributors

Both authors did the literature search, extracted and interpreted data, and wrote and revised the manuscript. MDC created the figures.

Declaration of interests

We declare no competing interests.

Acknowledgments

This work was supported by the National Institutes of Health.

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