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Antimalarial drug resistance in Africa: key lessons for the future

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Abstract

Drug-resistant parasites repeatedly arise as a result of widespread use of antimalarial drugs and have contributed significantly to the failure to control and eradicate malaria throughout the world. In this review, we describe the spread of resistance to chloroquine and sulfadoxine–pyrimethamine, two old drugs that are no longer used owing to high rates of resistance, and examine the effect of the removal of drug pressure on the survival of resistant parasites. Artemisinin-resistant malaria is now emerging in Southeast Asia in a unique and unexpected pattern. We will review the most recent genomic and clinical data to help predict the behavior of resistance to new antimalarial medications and inform strategies to prevent the spread of drug-resistant malaria in Africa in the future.

Keywords

malaria; drug resistance; artemisinin-based combination therapy; artemisinin resistance; molecular epidemiology; Africa

Introduction

Pathogen resistance to antimicrobials threatens the lives of people throughout the world. Malaria afflicts hundreds of millions of people each year, almost all of them are children in the nations with the most limited access to health care. Antimalarial resistance in this setting has dire consequences. Malaria treatment is based on guidelines that can be slow to change in response to resistance patterns and are not tailored to specific infection in the individual patient. When an antimalarial drug is failing, the clinical results are not immediately obvious. Even when efficacy is compromised, patients respond initially to the therapy, but within weeks the parasites recrudescence and the infection re-emerges. When access to health care is limited, a second bout of infection may not reach medical attention. Thus, even with mild, decreased clinical efficacy, drug resistance can occur and spread rapidly, which is reflected in increased malaria severity and mortality in sub-Saharan Africa. The spread of chloroquine resistance was a leading factor in the failure of the first malaria eradication campaign in the middle of the 20th century. Now, the emergence of resistance to currently

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Conflicts of interest

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used antimalarials is threatening the latest efforts to eliminate and, ultimately, eradicate malaria.

Drug pressure is one of the strongest selective pressures on the malaria genome, providing some of the most illustrative examples of *selective sweeps* in the scientific literature. During a selective sweep, linked neutral loci increase in frequency with advantageous alleles. These genomic “scars” can be used to identify loci responsible for drug resistance, as has recently been done with resistance to the artemisinins and other drugs.^{1–4}

In this concise review, we explore the dynamics of the emergence and spread of antimalarial drug resistance, as well as the fate of drug resistance as treatment policies change. Our goal is to identify key factors that influence the speed of the proliferation of drug resistance and to describe how current understanding of resistance to new antimalarial drugs may help to predict their future utility.

Resistance to chloroquine

Chloroquine acts by preventing detoxification of hemozoin within the parasite digestive vacuole.^{5,6} Resistance is caused by mutations within the *Plasmodium falciparum* chloroquine resistance transporter (PfCRT), with the mutation at position 76 (K76T) being particularly critical to the resistance phenotype.⁷ While the *pfcr* mutation leading to the K76T substitution is necessary for chloroquine resistance, it is not found in isolation and is part of a complex series of nine amino acid substitutions, which may be compensatory in nature. Examination of microsatellite loci flanking *pfcr* has indicated that chloroquine resistance has emerged at least four different times: once in Southeast Asia (spreading to Africa), once in Papua New Guinea, and twice in South America.⁸ The spread of chloroquine resistance required the emergence of a resistant strain of *Plasmodium* with adequate fitness to survive and propagate across continents in the context of widespread chloroquine use.

While the pattern of spread of chloroquine resistance and the presence of chloroquine use is clear, the removal of drug pressure in Southeast Asia, the South Pacific, and South America did not alter the high prevalence of chloroquine-resistant parasites. The presence of chloroquine-resistant parasites in Southeast Asia remains fixed at or near 100%, even in recently published studies.^{9,10} In South America, similar results have been seen in a variety of countries. In Venezuela and the Peruvian Amazon, chloroquine-resistant parasites remained fixed in the population after chloroquine use was replaced by artemisinin-based combination therapy (ACT).^{11,12}

Return of chloroquine sensitivity in Malawi

Despite the recognition of widespread chloroquine resistance in Africa, countries were reluctant to change treatment policy. In 1993, Malawi was the first African nation to change the first-line therapy from chloroquine to sulfadoxine–pyrimethamine (SP) owing to high rates of chloroquine treatment failure. Just 1 year after the removal of chloroquine, the proportion of infections from parasites with the critical PfCRT K76T mutation began to decline,¹³ was undetectable in Blantyre by 2001, and later was shown to be undetectable

nationwide.¹⁴ A clinical trial conducted in 2005, 12 years after the removal of chloroquine, estimated chloroquine efficacy to be 99%,¹⁵ and chloroquine susceptibility was maintained when it was used for repeated episodes of malaria in Malawi.¹⁶

The dramatic return of chloroquine efficacy by 2005 may have occurred through several different mechanisms: the spread of a highly fit susceptible genotype, just as chloroquine resistance spread; a back mutation in PfCRT position 76 to restore chloroquine sensitivity within the same genetic background; or the re-expansion of diverse chloroquine-susceptible parasites that survived a period of chloroquine drug pressure and increased in frequency after removal of the drug.

In a study published in 2010, we used microsatellites to examine the genomic regions flanking *pfcr*t in parasites before and after the drug policy change in Malawi and found that the return of chloroquine sensitivity was due to the resurgence of diverse chloroquine-sensitive parasites that had survived drug pressure, possibly in clinically immune hosts.¹⁷ These results suggested that there is a fitness cost to the parasite associated with *pfcr*t mutations in the absence of chloroquine pressure.

Drug resistance in Africa versus Asia

While chloroquine-resistant malaria was rapidly outcompeted after the removal of chloroquine as the first-line treatment in Malawi, a similar return of chloroquine-susceptible infections has not been observed in Asia. The uniqueness of this phenomenon in Africa, but not in Asia, is likely multi-factorial. Chloroquine use could never be eliminated in Asia or South America because it continued to be administered for the treatment of *Plasmodium vivax* infection. Thus, it remains available from both the formal and informal health sectors, and continues to be a source of drug pressure on *P. falciparum*. In contrast, there are years of demographic health surveys indicating that children in Malawi do not receive chloroquine.

Low malaria transmission in areas such as Asia and South America results in unique parasite population characteristics, including small effective population size and low diversity. Each affected individual receives one infectious bite from a single malaria genotype that is then taken up in a blood meal from another *Anopheles* mosquito. With a single parasite genotype in the mosquito midgut, there is no opportunity for genetic recombination to increase parasite diversity. Under these conditions, including smaller parasite populations, specific alleles can become fixed more quickly in low-transmission areas, leaving no drug-sensitive parasites, which can potentially survive, to expand with the removal of drug pressure. Resistance alleles to chloroquine are fixed in many Southeast Asian countries, as well as in some South American countries, making a return to sensitivity unlikely. In contrast, in higher transmission areas, there are more polyclonal infections and therefore greater opportunity for recombination and direct competition between different parasite strains.

Host immunity may also play an important role. Immunity to malaria is acquired with repeated exposure. People living in higher-transmission areas will also tend to have a higher degree of immunity, allowing for a larger reservoir of sensitive parasites in untreated,

clinically immune individuals. All these factors make Africa a more favorable environment for the return of sensitivity to drugs such as chloroquine.

Resistance to sulfadoxine–pyrimethamine

SP is a combination therapy that targets dihydrofolate reductase (PfDHFR) and dihydropteroate synthase (PfDHPS) within the folate biosynthetic pathway. Resistance is associated with the stepwise accumulation of mutations within the genes encoding these enzymes, including at codons 51, 59, 108, and 164 within *pfdhfr* and to codons 437, 540, and 581 within *pfdhps*.^{18,19} The more mutations accumulate in these genes, the greater the amount of resistance that is conferred to the parasite. For example, the S108N mutation confers some pyrimethamine tolerance to the parasite, whereas the triple mutant N51I/C59R/S108N is highly pyrimethamine resistant. Based on the examination of microsatellites flanking *pfdhfr*, the point mutation resulting in the DHFR S108N mutation occurs locally and relatively frequently, while the highly pyrimethamine-resistant triple mutant haplotype N51I/C59R/S108N appears to have emerged once in Southeast Asia, spreading into Africa.²⁰ A similar pattern was observed with DHPS, with highly resistant lineages emerging in Southeast Asia and South America, and then spreading from Southeast Asia to Africa.²¹

Like the pattern of the persistence of chloroquine-resistant malaria observed in Southeast Asia and South America, a high prevalence of DHFR and DHPS mutations have remained in some parasite populations in these regions even after the removal of SP from common use. For example, SP-resistant DHFR and DHPS mutants were found to be fixed in Venezuela 8 years after the removal of SP. These parasites were shown to have a single origin different from parasites in Asia and Africa.²²

Maintenance of SP resistance in Malawi

The utility of SP has also been severely compromised in Africa owing to widespread resistance. While chloroquine efficacy was estimated to be 99% in the clinical trial conducted in Blantyre in 2005, the efficacy of SP had declined to only 21%.¹⁵ By the time the first-line therapy was changed from SP to an ACT in 2007, the prevalence of the highly resistant DHFR triple mutant N51I/C59R/S108N and DHPS double mutant A437G/K540E was >95%. This prevalence remained high in 2012, 5 years after the switch from SP to an ACT, and was high in Blantyre, as well as in two other rural sites with higher malaria transmission. In addition, the prevalence of the DHPS triple mutant 437G/540E/581G, which is associated with failure of intermittent preventive therapy in pregnant women,²³ increased from 0% in 1999 to 4% in 2012.²⁴ Examination of selective sweeps before, during, and after the switch from SP to an ACT showed little change in sweep characteristics over time.

In contrast to chloroquine, these results suggest little to no fitness cost of SP-resistance mutations in the absence of drug pressure in this epidemiological setting. Alternatively, the continued use of SP for intermittent preventive therapy in pregnant women and/or the use of trimethoprim/sulfamethoxazole in HIV-positive individuals could have resulted in some maintenance of drug pressure, even after the change in drug policy. However, to date there

is no evidence that trimethoprim/sulfamethoxazole has any impact on the prevalence of SP-resistant alleles, or that SP resistance affects trimethoprim/sulfamethoxazole efficacy.^{25,26} In addition, Iriemenam *et al.* found the impact of SP-IPTp (intermittent preventive treatment in pregnancy) on the increase of SP-resistant parasites in pregnant women to be minor compared to the use of SP for case management in the general population.²⁷ Thus, these are likely not the sole factors responsible for the sustained high prevalence of SP-resistant mutants in Malawi.

Future of former first-line antimalarials in Africa

Current data from Malawi indicate little to no fitness cost of SP-resistance mutations to the parasite, suggesting that SP sensitivity is not likely to return in epidemiologically similar geographic regions in the near future, especially with the continued use of SP for intermittent preventive therapy. The effectiveness of intermittent preventive therapy in pregnant women is also threatened by the emergence of the DHPS 437G/540E/581G triple mutant in Malawi and other areas. Therefore, in areas like Malawi, where SP resistance is high, this drug may not have future use.

In contrast, chloroquine sensitivity is likely to return to sub-Saharan Africa as ACTs remove chloroquine selective pressure. This is now being observed throughout the region.^{28–32} In addition to the reduction of chloroquine drug pressure, the lumefantrine component of artemether/lumefantrine, one of the most widely used ACTs, may select for chloroquine-sensitive alleles, increasing the likelihood of the return of chloroquine sensitivity in areas where these ACTs are used and where susceptible parasites still exist.³³

If chloroquine-susceptible malaria returns throughout sub-Saharan Africa, it may play an important role in malaria prevention and treatment in the future. Because chloroquine has a known pharmacokinetic profile, a validated molecular marker for resistance, and an excellent safety profile, this information can be used to develop rational drug-combination therapies to prevent the emergence or re-emergence of chloroquine resistance. While a chloroquine combination may be used for treatment of malaria disease in the future, it is even more appealing as an option for prevention in vulnerable populations such as pregnant women or young children because it is safe in all trimesters, has dosing regimens for all age groups, and has a long elimination half-life and provides protection for 4–6 weeks after the dose is administered.

Resistance to artemisinins

Artemisinin derivatives are fast-acting, well-tolerated drugs that are often paired with longer-acting partner drugs as ACTs, which are now the first-line treatment for *P. falciparum* throughout the malaria-endemic world. Artemisinin resistance has emerged in Southeast Asia, manifesting itself as delayed clearance of parasitemia following treatment with artemisinin derivatives.^{34–37} Recently, this resistance has been shown to be associated with mutations within a kelch protein located on *P. falciparum* chromosome 13 (K13 propeller),^{38,39} and secondary loci may also be involved.³ K13 propeller mutations have been associated with delayed parasite clearance in several Southeast Asian countries,

including Cambodia, Vietnam, and Myanmar, and have been shown to have spread between countries as well as to have emerged independently in different countries.^{3,40}

Artemisinin resistance in Africa

Monitoring of parasite clearance rates following treatment with ACTs has occurred at various sites within Africa. Overall, *P. falciparum* parasites seem to clear rapidly in most African countries sampled to date.^{40,41} In contrast, a single study by Borrmann *et al.* reported increased rates of day-1 parasitemia and increased risk of parasite recrudescence following treatment with ACTs from 2005 to 2008. While the hints of delayed clearance observed in the Borrmann study could suggest decreased susceptibility to ACTs, they could also reflect decreased clinical immunity within the study population.⁴²

Despite rapid clearance of parasites in all African studies conducted thus far, K13 propeller mutations have been observed at low levels in parasites from African study sites.^{43,44} Many of these mutations are within the propeller region of K13, but are different from the mutations observed in Southeast Asia, and occur at low frequencies, often within a single study site. The presence of K13 propeller mutations within Africa, where parasite clearance is generally rapid, suggests that not all K13 propeller mutations are associated with resistance, that secondary loci are involved in resistance and found in Asia but not in Africa thus far, or that the resistance phenotype is masked by high levels of antimalarial immunity.

The implications for the spread or emergence of artemisinin resistance in Africa are complex. The independent emergence of resistance in Myanmar (in contrast to spread from Cambodia) and the presence of existing K13 propeller mutations in African countries highlight the possibility of independent emergence of resistance in Africa. However, if secondary and/or background mutations are required to augment resistance and these are not found in African parasites, then the level of resistance conferred by K13 propeller mutations may be less than that observed in parasites with an Asian genetic background. If K13 mutations on an Asian genetic background were to spread to Africa, any association with secondary loci on different chromosomes could be quickly unlinked as parasites reproduce with diverse African parasites. Thus, it is possible that it may be more difficult for artemisinin resistance to become established in Africa compared to Asia, although this remains to be seen. Further research is needed to understand the role of African K13 propeller mutations in artemisinin resistance.

Conclusions

The emergence and spread of antimalarial drug resistance has been one of the primary obstacles to malaria control in areas with the highest burden of infection and disease. Characterization of resistance to chloroquine and SP, drugs that were initially used for the treatment of malaria, occurred late, after resistance had spread globally and there was no opportunity for containment. The emergence of resistance to artemisinins in Southeast Asia offers an unprecedented opportunity to proactively avert spread to malaria-endemic Africa, and the Global Plan for Artemisinin Resistance Containment (GPARC), established by the World Health Organization, is being implemented for this purpose. However, the ability of

the candidate marker of artemisinin resistance to emerge on a variety of backgrounds may mean that artemisinin resistance will emerge in Africa regardless of containment efforts in Asia.

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