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ROLL BACK OF *PLASMODIUM FALCIPARUM* ANTIFOLATE RESISTANCE BY INSECTICIDE-TREATED NETS

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Preserving efficacy of the limited arsenal of antimalarial drugs is of critical importance to public health in malaria-endemic regions. Of particular concern is the vulnerability of those agents that are most affordable, chloroquine and the antifolate duo, sulfadoxine/pyrimethamine (S/P), or second-generation chlorproguanil/dapsone (LAPDAP). On page 238 of the current issue, Alifrangis and others report that an increased frequency of *Plasmodium falciparum* strains carrying the wild-type dihydrofolate reductase (*dhfr*) the allele was observed following the use of insecticide-treated nets (ITNs), and suggest that their approach might help to restore sensitivity to S/P.

The study of interest was conducted in two neighboring Tanzanian villages, Magoda and Mpapayu, from 1998 to 2000, at a time when complex *dhfr* and *dhps* mutation frequencies exceeded 60% and fully wild-type haplotype frequencies ranged from 0% to 5%. Of particular interest, the majority *dhfr* polymorphism was the triple mutant (108N/51I/59R); data on the quadruple mutant haplotype containing 164L was not reported. ITNs were provided to all households in Magoda, but were not distributed in Mpapayu. In addition to *dhfr/dhps* polymorphisms, children between the ages of 0.5 and 5 years from both villages were studied for *P. falciparum* infection and anemia. During the ITN trial, S/P was used as the first-line antimalarial in both villages. At the conclusion of their study fully wild-type *dhfr* allele frequencies were approaching 20% in Magoda, but remained unchanged in Mpapayu. The investigators conclude that their results are consistent with the Mackinnon/Hastings model,[1] in which the spread of antimalarial drug resistance is constrained by reducing transmission through the use of ITNs.

These results appear at an important point in the history of antifolate treatment of malaria and other microbial pathogens. Recent reviews[2–7] have suggested that malaria control efforts face a pending disaster if S/P resistance takes hold in African *P. falciparum* as it has in Southeast Asia and South America. Numerous familiar factors underlie this extreme concern. The majority of annual malaria cases and deaths occur in Africa in association with *P. falciparum* infection. Public health in many malaria-endemic African countries is highly stressed by human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome and tuberculosis. With widespread chloroquine resistance in *P. falciparum,* antifolates are the last inexpensive antimalarial drugs remaining.

Additional factors specific to S/P efficacy and evolving *dhfr/dhps* polymorphism intensify the concern focused on *P. falciparum* antifolate resistance. S/P resistance arises quickly when this drug strategy is used by itself and is best understood to result from the relatively long persistence of the drug in the blood (up to 52 days following treatment).[4] Significant

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multinational effort[8] has been invested in development of the alternative antifolate combination drug, LAPDAP, that exhibits rapid clearance parameters (<1 day).[9] Field studies have shown that LAPDAP is effective against *P. falciparum* carrying the triple mutant haplotype described above,[10] and is not associated with an increase in *dhfr* mutations closely associated with antifolate resistance.[11] However, a critical "line in the sand" is crossed if the L164 mutation is added to the N108/I51/R59 *dhfr* haplotype because parasites carrying the quadruple mutant haplotype are both S/P and LAPDAP resistant.[12,13] The simple remedy to reduce the selective pressure bearing down on *P. falciparum* sensitivity to antifolate drugs would appear to involve heavy restrictions on the use of antifolates that exhibit limited efficacy against this troublesome parasite. Here an unfortunate coincidence arises because the antifolate combination trimethoprim-sulfamethoxazole (co-trimoxazole), which is weakly effective against *P. falciparum,*[14] has recently been recommended for routine use in HIV-positive people in Africa to combat opportunistic infections;[15,16] malaria researchers quickly challenged the merits of this action.[17] To stabilize the antifolate malaria drugs and the fragile antimalarial arsenal, significant attention is turning to consider treatment strategies that include a third drug.[2,6,18] Artesunate and chloroquine both exhibit rapid killing of malaria parasites and would limit parasite exposure to S/P.[6] Whereas artesunate would be the more effective anti-malarial agent, an artesunate plus S/P combination may not be affordable. Chloroquine is noted to remain effective against some *P. falciparum* strains (sensitive, RI, and RII) and would be much less expensive.[6]

Within this matrix of limited antimalarial treatment options, fleeting time to act, and threatened public health, the results of Alifrangis and others may provide an elegant new alternative to more cumbersome (development of new drug combinations for regulatory approval) or risky (ineffective partner drugs) combination drug strategies. Consistent with the combination strategies and models to limit the spread of drug-resistant parasites, ITNs reduce transmission and thereby reduce the number of drug-exposed parasites. At the very least, ITNs in combination with LAPDAP might help to avert emergence of the 164L *dhfr* mutation and significant dismantling of efforts to develop new and rationally based antimalarial treatment strategies.

As further ITN and drug combination studies are conducted in regions where antifolate-resistant *P. falciparum* is highly prevalent, it will be interesting to assess not only the return of parasites carrying antifolate sensitive alleles, but also to evaluate the persistence of the resistant alleles. Recent studies have shown that cessation of chloroquine treatment within malaria-endemic regions is followed quickly by a significant reduction in alleles conferring chloroquine resistance (*pfcrt* 76T; *P. falciparum* chloroquine resistance transporter).[19,20] These results suggest that the *pfcrt* 76T allele carries a fitness liability in the absence of chloroquine, and that it may be possible to restore chloroquine's useful therapeutic life. The use of S/P was abandoned in the mid 1980s in Southeast Asia due to loss of efficacy, yet molecular epidemiologic surveys conducted as recently as this past year report high frequencies of the *dhfr* triple and quadruple haplotypes associated with S/P and S/P plus LAPDAP resistance.[21–24] These results, together with those presented by Alifrangis and others, cause one to wonder if strategies might be developed to stabilize the efficacy of the affordable antimalarial antifolate drugs.

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