The role of mutations in uncomplicated *Plasmodium falciparum* malaria and sulfadoxine pyrimethamine efficacy in Mpumalanga Province, South Africa.

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ABSTRACT

The antifolate combination of sulfadoxine and pyrimethamine (SP) is one of few remaining affordable drug combinations available for wide-scale treatment of uncomplicated *Plasmodium falciparum* malaria in Africa. *In vivo* studies of SP efficacy conducted during 1998, 2000 and 2002 at the Naas sentinel site in Mpumalanga province, South Africa, demonstrated a gradual non-significant increase in late treatment failure (LTF) and early treatment failure (ETF) resistance to SP, while gametocyte carriage increased significantly between 1998 and 2002 ($p < 0.0001$). This study aimed to determined and compare the frequency of dihydrofolate reductase (*dhfr*) and dihydropteroate synthetase (*dhps)* resistant haplotypes in *P. falciparum* parasites from patients treated with SP in three consecutive standardized *in vivo* therapeutic efficacy studies in Mpumalanga province, since implementation of SP as first line treatment in 1998, and to investigate associations between the presence of mutations and treatment outcomes after SP treatment. Four hundred-and-three samples were studied and 358 yielded polymerase chain reaction products. A novel high throughput sequence-specific oligonucleotide probe-based approach was used to examine the resistance status of the three *in vivo P. falciparum* populations. Screening for the presence of all known point mutations in *dhfr* and *dhps* genes revealed that only five *dhfr* and three *dhps* allelic haplotypes were present. In all the samples investigated, point mutations were identified only at codons 108, 51 and 59 of the *dhfr* gene and at codons 347 and 540 of the *dhps* gene. The prevalence of *dhfr* resistant haplotypes was 35.4% in 1998, 38.7% in 2000, and 41.0% in 2002, while the prevalence of *dhps* resistant haplotypes was 9.7% in 1998, 7.2% in 2000 and 41.6% in 2002, the latter representing a significant increase ($p < 0.002$). The prevalence in both *dhf*r and *dhps* gene resistant haplotypes were selected gradually during the three *in*

vivo studies in Mpumalanga province. Infection with parasites having triple *dhfr* mutations and double *dhps* mutations, "the quintuple mutant", was associated with SP treatment failure ($p < 0.001$). Mutations at both *dhfr* and *dhps* loci may be important predictors of SP resistance in Mpumalanga province.