Reviewer's report

Title: Tracing the origins and signatures of selection of antifolate resistance in island populations of Plasmodium falciparum

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Reviewer: Hamza A Babiker

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Major points

The authors have examined SNPs in dhfr and dhps and microsatellites flanking the two genes and showed a pattern of mutations in dhfr and dhps leading to SP resistance in a P. falciparum population in Sao Tome and Principe Islands, off the cost of West Africa. They concluded that their findings provides additional evidence for the crucial role of gene flow and drug resistance selective pressure in the rapid spread of SP resistance in P. falciparum populations.

A major problem with the interpretation of their data is that they can not rule out the local demography and parasite population structure (e.g. linkage due to inbreeding), this is evident as neutral MS loci showed significant FST values similar to MS flanking dhps. Therefore, the linkage and differentiated seen between the examined loci can be driven by the genetic structure of the parasites (inbreeding and/or population expansion). It would be difficult to infer signature of selections or linkage disequilibrium among such parasite population. Similar linkage between microsatellite haplotypes for dhfr and dhps genes have been observed in South America, were crossing and recombination is limited (e.g. see McCollum AM, et al 2007, Antimicrob Agents Chemother. 51:2085-2091 also Cortese et al 2002 J Infect Dis. 186:999-1006).

The authors have done a great amount of work, however there is very little new in this manuscript, a large number of studies have demonstrated a common origin for highly resistant pyrimethamine allele across Southeast Asia and at different sites in Africa, as cited by the authors. In addition low frequency lineages for the triple mutant (N51I/C59R/S108N) dhfr, other than the one suspected to be of South East Asian origin, have been documented in many areas highlight the role of local evolution as a result of crossing and recombination. Furthermore, the genetic signature for selective pressure of SP on P. falciparum populations have been well documented in form, of linkage between drug resistance genes and lower heterozygosity (shape of the curve of variation) around dhfr and dhps genes (e.g. McCollum et al 2008 and Pearce et al 2009) as cited by the authors.

Minor points

Page 5, Results section, line 3, change the word "frequency" with "prevalence" as gene frequency is difficult to estimate on natural P. falciparum infection, due to
presence of multiple infections

Page, 6 section dhfr and dhps haplotype characterization, line 4, what is number of samples that have been used to construct 23 haplotypes of dhfr and 9 haplotypes of dhps?

Page 6, second para, and Table 1. haplotype H2 with double and triple mutations is not identical, these are different haplotypes and should be separated, a haplotype is "combination of alleles at multiple loci that transmit together". It seems that the authors have only detected 5 haplotypes for dhfr (not 23) and 8 (not 9) for dhps

Page 9, discussion, para 2, divide of H2 haplotype into 2 of double and triple mutations.

Delete table 6. Information in this table can be included in the text.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.