

## **Spread of a single multidrug resistant malaria parasite lineage (*Pf* Pailin) across the Greater Mekong subregion**

The spread of artemisinin resistance in *Plasmodium falciparum* and the subsequent loss of partner antimalarial drugs in the Greater Mekong subregion (GMS)<sup>1</sup> represents one of the greatest threats to the control and elimination of malaria. Artemisinin resistance is associated with mutations in the *Pfkelch* gene. Initially multiple independent kelch mutations were observed<sup>1</sup>, but in a recent sinister development, a single dominant artemisinin-resistant *P. falciparum* C580Y mutant lineage has arisen in Western Cambodia, outcompeting the other resistant malaria parasites, and subsequently acquiring resistance to piperaquine<sup>2</sup>. Cambodia had adopted dihydroartemisinin-piperaquine as first line antimalarial treatment in 2010, but has now been forced to switch its first line artemisinin combination treatment (ACT) back to artesunate-mefloquine as a consequence<sup>3</sup>. This dominant multi-drug resistant parasite lineage, identified first in Pailin in Western Cambodia ("*Pf* Pailin"), then spread to North-Eastern Thailand and Southern Laos<sup>2</sup>. We now find that this same *Pf* kelch C580Y lineage had reached the south of Vietnam by 2011. Microsatellite typing of 96 *P. falciparum* isolates from the Binh Phuoc locality in 2011-2012 showed that 6% of isolates from patients with acute malaria had the same *Pf* Pailin flanking sequence surrounding the *Pf* kelch C580Y gene as that observed in parasites from the affected areas of the other three GMS countries<sup>2</sup>. Other *Pf* kelch mutations and flanking sequences were found in 32% (31/96) of isolates. However by 2016 in Binh Phuoc the *Pf* Pailin lineage had outcompeted all other parasites, and now accounted for nearly all *P. falciparum* isolated (94%; 85/90), and it had acquired piperaquine resistance (evidenced by amplification in the *Pf* plasmepsin 2 gene). This multi-drug resistant *Pf* Pailin lineage is responsible for an alarming rise in dihydroartemisinin-piperaquine treatment failure in this area with recrudescence rates now exceeding 50%<sup>4</sup>. The evolution and subsequent transnational spread of this single fit multidrug resistant malaria parasite lineage is of international concern.

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### Legend to Figure

The artemisinin resistant *P. falciparum* C580Y lineage was detected first in Pailin, Western Cambodia, in 2008<sup>2</sup>. *Pf* Pailin spread east and later acquired piperaquine resistance. This multi-drug resistant lineage has reached the south of Vietnam now encompassing all four countries of the Eastern Greater Mekong subregion. On the left, the microsatellite genotyping is shown covering - 247 kb to +269 kb either side of the *Pf* kelch gene (*Pf* kelch C580Y mutant: red, wild type: lighter

blue). Each row represents one parasite isolate; white cells indicate identical microsatellite alleles compared with the most frequent allele and dark blue cells indicate differences. All 277 genotyped Pf Kelch C580Y mutated parasites showed the same long haplotype, except 6 isolates from Kayin<sup>2</sup> (Myanmar).

