Molecular epidemiology of resistance to antimalarial drugs in the Greater Mekong subregion: an observational study

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Summary

Background The Greater Mekong subregion is a recurrent source of antimalarial drug resistance in Plasmodium falciparum malaria. This study aimed to characterise the extent and spread of resistance across this entire region between 2007 and 2018.

Methods P falciparum isolates from Myanmar, Thailand, Laos, and Cambodia were obtained from clinical trials and epidemiological studies done between Jan 1, 2007, and Dec 31, 2018, and were genotyped for molecular markers (pfk1ch, pfcr, pfplasmepsin2, and pfmdr1) of antimalarial drug resistance. Genetic relatedness was assessed using microsatellite and single nucleotide polymorphism typing of flanking sequences around target genes.

Findings 10632 isolates were genotyped. A single long pfk1ch Cys580Tyr haplotype (from -50 kb to +31·5 kb) conferring artemisinin resistance (PfPailin) now dominates across the eastern Greater Mekong subregion. Piperaquine resistance associated with pfplasmepsin2 gene amplification and mutations in pfcr downstream of the Lys76Thr chloroquine resistance locus has also developed. On the Thailand–Myanmar border a different pfk1ch Cys580Tyr lineage rose to high frequencies before it was eliminated. Elsewhere in Myanmar the Cys580Tyr allele remains widespread at low allele frequencies. Meanwhile a single artemisinin-resistant pfk1ch Phe446Ile haplotype has spread across Myanmar. Despite intense use of dihydroartemisinin–piperaquine in Kayin state, eastern Myanmar, both in treatment and mass drug administrations, no selection of piperaquine resistance markers was observed. pfmdr1 amplification, a marker of resistance to mefloquine, remains at low prevalence across the entire region.

Interpretation Artemisinin resistance in P falciparum is now prevalent across the Greater Mekong subregion. In the eastern Greater Mekong subregion a multidrug resistant P falciparum lineage (PfPailin) dominates. In Myanmar a long pfk1ch Phe446Ile haplotype has spread widely but, by contrast with the eastern Greater Mekong subregion, there is no indication of artemisinin combination therapy (ACT) partner drug resistance from genotyping known markers, and no evidence of spread of ACT resistant P falciparum from the east to the west. There is still a window of opportunity to prevent global spread of ACT resistance.

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Research in context

Evidence before this study
We searched PubMed on Dec 18, 2019, using the terms “artemisinin resistance” or “piperaquine resistance”, and “genotype”, and either “Cambodia”, “Thailand”, “Myanmar” or “Laos” without any date or language restrictions. This search identified 36 non-duplicate articles, of which 26 contained information on the molecular epidemiology of artemisinin-resistant or piperaquine-resistant Plasmodium falciparum. Several articles described the finding of multiple different pfkelch13 mutations conferring artemisinin resistance in P falciparum malaria parasites isolated across the Greater Mekong subregion, with reports by Imwong and colleagues and Amato and colleagues describing the emergence of a dominant parasite lineage in the eastern Greater Mekong subregion (comprising eastern Thailand, Cambodia, Laos, and the Southern Vietnam geographical region). pfplasmepsin2 gene amplification and pfcrt mutations linked with piperaquine resistance and high rates of treatment failure were associated with this same lineage. The possibility of resistance spreading from the eastern to the western Greater Mekong subregion (comprising western Thailand and Myanmar), the spread of resistant lineages within Myanmar, and the role of intensive dihydroartemisinin–piperaquine deployment, particularly in mass drug administrations, in the selection of antimalarial drug resistance has not been explored.

Added value of this study
This very large molecular epidemiology study done over 12 years describes the evolution and spread of antimalarial drug resistance across the entire Greater Mekong subregion. It shows that multiple soft selective sweeps with different pfkelch mutant (artemisinin resistant) parasite lineages have been followed by hard selective sweeps by presumably fitter parasites. These increasingly dominant lineages have emerged in Myanmar and the eastern Greater Mekong subregion, but they differ from one another, and there is no evidence of spread from the eastern to the western Greater Mekong subregion. There is also no evidence for piperaquine resistance in the western Greater Mekong subregion. Intense targeted elimination activities using dihydroartemisinin–piperaquine in the treatment of symptomatic malaria and mass treatments have been successful and did not select for resistance.

Implications of all the available evidence
Outside the eastern Greater Mekong subregion there is still a window of opportunity to halt the spread of artemisinin resistance westward to India and Africa by using dihydroartemisinin–piperaquine in targeted malaria elimination activities.

Methods

Study design and participants
As part of studies11,13–25 on the epidemiology, treatment, and targeted elimination of artemisinin-resistant malaria done between Jan 1, 2007, and Dec 31, 2018, across the Greater Mekong subregion, venous blood samples, filter paper blood spots, and completed diagnostic test strips were collected from patients presenting with microscopy or rapid test-confirmed uncomplicated falciparum malaria and from healthy participants in surveys of villages where targeted malaria elimination activities were planned (appendix pp 10–11).

Procedures
DNA was extracted from either dried blood spots, completed malaria rapid diagnostic test strips, or frozen whole blood samples, by standard methods at the Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand. DNA was purified with QIAamp DNA Mini kits (Qiagen; Düsseldorf, Germany). Polymorphism in the pfkelch gene was examined by nested PCR amplification covering the propeller region of the gene and Sanger sequencing (ABI Sequencer; Applied Biosystems). The sequences were aligned against the 3D7 reference strain pfkelch gene (putative PF13_0238; NCBI sequence XM_001350122.1) using Bioedit software. pfcrt was amplified from the DNA template using nested PCR covering exons 1 and 2 (amino acids 1–120) and Sanger sequenced. A restriction fragment length polymorphism assay was developed to assess previously identified pfcrt mutations:22,25 Phe145Ile, Ile218Phe, Asn326Ser, Met343Ile/Leu, and Gly353Val.

Nine microsatellite markers and five single nucleotide polymorphisms (SNPs) in the flanking regions each side of the pfkelch gene spaced from –56 kb to +225 kb were
assessed. The microsatellite PCR generated product lengths were compared with internal size standards (Genescan 500 LIZ) on an ABI 3100 Genetic Analyzer (Macrogen, Seoul, South Korea). Genescan and Genotyper software was used to measure allele lengths and quantify peak heights. SNPs were examined by PCR amplification15 on chromosome 14 (appendix pp 2, 13–14). A nested PCR was developed to identify the precise position of the pfplasmepsin2 amplicon chromosomal breakage points. Primer pairs amplified across unique junctions of multiple copy pfplasmepsin2 but produced no product in samples with a single copy (appendix p 15).

To identify the size and gene content of the amplified chromosomal regions, we developed 37 real-time PCR assays to measure the copy numbers of genes covering −61 kb at 5’ to +130 kb at the 3’ end of pfplasmepsin2 on chromosome 14 (appendix pp 2, 13–14). A nested PCR was developed to identify the precise position of the pfplasmepsin2 amplicon chromosomal breakage points. Primer pairs amplified across unique junctions of multiple copy pfplasmepsin2 but produced no product in samples with a single copy (appendix p 15).

### Statistical analysis

Genetic variation at each microsatellite locus or expected heterozygosity (Hₑ) was assessed using Hₑ = (n²/(n−1)) (1–Pₑ^2), with sampling variance defined as 2n/[(n−1)²(n−2)]Pₑ^2(1−Pₑ^2), where n is the sample size and Pₑ the frequency of the allele at position i. Heterozygosity at each location was compared with wild-type sequences.

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**Figure 1:** Frequency distributions of mutations found in the pfKelch gene in Plasmodium falciparum isolates obtained from four countries of the Greater Mekong subregion, 2007–18.
using Fisher’s least significant difference and Mann-Whitney U tests. Proportions were compared with χ² or Fisher’s exact test.

To order the isolates by similarity, we computed pairwise identity-by-state, defined as the proportion of identical alleles over all markers typed (not missing for both isolates). For markers where two or more alleles were observed, phased haplotypes were not estimated but instead identity-by-state was called if at least one allele was shared in both isolates. Hierarchical agglomerative clustering was applied to the distance matrix, where the pairwise distance was defined as one minus the proportion identical-by-state. The output isolate ordering was used to plot the observed marker data using previously published software.26

Role of the funding source
The funders of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results
14509 positive samples were obtained (appendix pp1, 10–11). 10632 samples had sufficient DNA for genotyping, of which 2420 (23%) were from used rapid diagnostic tests, 4912 (46%) were from filter paper dried blood spots (DBS), 3097 (29%) were from frozen packed red cell samples, and 203 (2%) were from frozen whole blood samples.

5633 P. falciparum isolates collected between Jan 1, 2007, and Dec 31, 2018, were sequenced for pf kelch mutations. Two genotypes, the Cys580Tyr mutation in the eastern Greater Mekong subregion and, to a lesser extent, the Phe446Ile mutation in Myanmar, have come to predominate with a corresponding decline in the proportions of other propeller mutations. The proportion of Cys580Tyr isolates has increased.15 Cys580Tyr was present in three (33%) of nine of isolates in 2007 compared with 18 (90%) of 20 in 2017 (p=0.0043; figure 1). Within Myanmar, the Cys580Tyr mutation was distributed widely at low frequencies in Kayin (177 [5%] of 369), in northwestern Thailand along the Myanmar border (figure 1; appendix pp 3–4). In western Cambodia, the Cys580Tyr allele was found also in Ranong, southwestern Thailand adjacent to Myanmar (21 [28%] of 74), and in Mae Hong Son (two [15%] of 13), and Mae Sot (39 [11%] of 369), in northwestern Thailand along the Myanmar border (figure 1; appendix pp 3–4). In western Cambodia the proportion of Cys580Tyr isolates has increased.15

Cys580Tyr was present in three (33%) of nine of isolates in 2007 compared with 18 (90%) of 20 in 2017 (p=0.0043; figure 1). Within Myanmar, the Cys580Tyr mutation was distributed widely at low frequencies in Kayin (177 [5%] of 369), Kachin (one [1%] of 100), and Sagaing (six [10%] of 58) states. The Phe446Ile allele was observed only in the western Greater Mekong subregion (figure 1). The prevalence of Phe446Ile among the P. falciparum isolates was highest towards the north of Myanmar.15 Phe446Ile was also common inside Kayin state but prevalence was lower at the Thai border, and it was not found in 2014 in Ranong, Thailand, near the southern tip of Myanmar.

Comparison of the Cys580Tyr flanking sequences (n=113) with contemporary pf kelch wild-type isolates (n=95) in the western Greater Mekong subregion between 2007 and 2017 revealed a marked reduction in the heterozygosity of mutant infections over time (H, 0.220, standard error [SE] 0.002) suggesting a selective sweep (figure 2). In the subset of Cys580Tyr isolates from 2016

Figure 2: Polyallelic marker data from all Cys580Tyr mutant Plasmodium falciparum isolates
(A) Data from all mutant isolates from Myanmar (n=113) with matched wild-type isolates (n=95). (B) Data from all mutant isolates from western Cambodia (n=189) with matched wild-type isolates (n=87). Shown are data at position 580 on the pf kelch gene (0 on the x-axis) and in an interval from -56 kb to +225 kb surrounding the pf kelch gene. The colours correspond to the different alleles, whereby an independent colouring scheme was applied to each polyallelic marker separately. The colour scheme is based on all observed alleles for all samples from the Greater Mekong subregion (not only those shown in this figure), so that comparisons can be made across figures. When multiple alleles were observed at a single locus, the column is broken into subcolumns with the corresponding colours. White corresponds to missing data. For the pf kelch gene, green is wild-type and red is Cys580Tyr. The number of distinct alleles observed in all the data for each marker is given by the number in parentheses.
and 2017 (n=74) the sweep was more evident, with a mean $H_s$ of 0.130 (SE 0.001) versus 0.696 (0.001) in contemporary wild-type infections (p<0.0001). Flanking sequence variation was diminished for about 50 kb either side of the Cys580Tyr $pfkelch$ gene (appendix pp 3–4).

Comparison of the flanking haplotypes showed that the Cys580Tyr allele in the northwest (Mae Hong Son, n=2) and southwest of Thailand (Ranong, 1000 km to the south, n=13) shared a common origin with the Cys580Tyr alleles observed in northwestern Myanmar (Sagaing, 1800 km north of Ranong, n=6) and eastern Myanmar (Kayin, n=47). This haplotype has not dominated except on the Thai border, where a focus has been eliminated by targeted elimination activities. This common haplotype was genetically remote from the previously characterised PfPailin Cys580Tyr haplotype, which has spread across the eastern Greater Mekong subregion (figures 2, 3). Reduced flanking sequence variation ($H_s < 0.1$) was observed in a smaller segment of the genome surrounding Cys580Tyr in Myanmar in 2016–17 than in earlier years (2010–15), suggesting continued recombination after the initial selection event.

Flanking sequences around the $pfkelch$ Phe446Ile allele were assessed in 202 samples from Myanmar obtained between Jan 1, 2014, and Dec 31, 2017. In 2014–16, at least two $pfkelch$ Phe446Ile haplotypes were observed in Kayin, Kachin, and Sagaing states (n=90), but by 2017, one predominated and had spread across more than 1000 km from the northwest to the east of Myanmar (Sagaing n=13; Kayin n=99) with a mean $H_s$ of 0.207 (SE 0.066; appendix p 5). The size of the region of the genome around the Phe446Ile and Cys580Tyr alleles, in which diversity was reduced, was roughly similar (ie, about 50 kb either side of the gene). By comparison, diversity around the 446 locus in the 95 $pfkelch$ contemporary wild-type infections from the same locations was high (mean $H_s$ 0.696, SE 0.001).

Between Jan 1, 2007, and Dec 31, 2018, $pfplasmepsin2$ amplification was considerably more frequent in the eastern Greater Mekong subregion (n=4571 samples; median copy number 1.83, range 1.60–2.76) than in Myanmar where it was found only in Kyain Seikgyi, Kayin state (2015–17; 51 [1%] of 4221; figure 4). To investigate possible importation from the eastern Greater Mekong subregion, the amplicons and their breakpoint junction sequences were compared. The amplicon containing multiple-copy $pfplasmepsin2$ genes...
in Pailin (west Cambodia), characterised previously, was around 18 kb in length and carried three genes: \textit{pfplasmepsin1}, 2, and 3. None of the 36 isolates from Myanmar with amplified \textit{pfplasmepsin2} genes had the same breakpoint as the Cambodian isolates and the amplicons varied in length from 81 kb to more than 190 kb. These findings suggest independent origins for \textit{pfplasmepsin2} amplification in Myanmar and provide no evidence for selection or importation from the eastern Greater Mekong subregion.

Flanking sequences around the \textit{pfplasmepsin2} gene in 51 multiple-copy isolates and 54 single-copy isolates from the eastern Greater Mekong subregion were compared (appendix p 7). There was reduced diversity in the eastern Greater Mekong subregion (mean $H$, 0·271, SE 0·005), compared with high diversity in wild-type isolates (mean $H$, 0·608, SE 0·034; p <0·0001; appendix pp 8–9). In Myanmar, there was no evidence of a selective sweep; flanking sequences around multiple-copy \textit{pfplasmepsin2} isolates from Kayin states (n=10) showed different haplotypes (appendix pp 8–9), suggesting different origins (H, 0·643, SE 0·057) and providing no evidence of selection by the intensive use of dihydroartemisinin–piperaquine in mass treatments.

Between Jan 1, 2007, and Dec 31, 2018, 6984 \textit{P falciparum} isolates were assessed for \textit{pfmdr1} amplification. Amplified \textit{pfmdr1} was found across the region (figure 5); in Mandalay eight [13\%] of 59 in 2013–14 and Kayin, 79 [3\%] of 2139 in 2017) in Myanmar and on the northwestern border of Thailand in Mae Sot (19 [3\%] of 62 in 2015). This figure for Mae Sot is slightly lower than the proportion in 1993–94 in the same area (30 [48\%] of 62) when mefloquine monotherapy was first-line treatment. Amplified \textit{pfmdr1} amplification was also found in southern Laos (seven [4\%] of 173 in 2018), and Stung Treng, northern Cambodia (seven [8\%] of 89 in 2018), and in northeastern Thailand (Ubon Ratchathani and Srisket) near the Cambodian border (one [7\%] of 13 in 2016).

With regard to submicroscopic \textit{P falciparum} parasitaemias, the proportion of isolates with \textit{pfplasmepsin2} amplification was low or zero before (n=142) and after (n=38) mass treatment with
Resistance occurred, but hard selective sweeps by presumably fitter parasites have gradually replaced the now spread across the eastern Greater Mekong subregion, comprising northern Laos and the main body of Thailand, with borders with Yunnan province, China, northeast India, and Bangladesh. The area between these two regions, comprising Myanmar and adjacent western Thailand, with borders with southern Vietnam, southern Laos, and northern Cambodia, is largely malaria free. In the eastern region, except for Laos, dihydroartemisinin–piperaquine has been used extensively for many years. A single long haplotype of CVIET at residues 72–76; figure 6; appendix p 15),10–13,27 There was also no evidence for further selection of pfkelch mutations after mass treatment.

Discussion

Antimalarial drug resistance in P. falciparum continues to evolve in the Greater Mekong subregion, making the treatment of falciparum malaria increasingly difficult and threatening national and regional malaria elimination targets. Artemisinin resistance, first recognised in 2007 in western Cambodia, is closely associated with mutations in the pfkelch gene (chromosome 13) propeller region.17 Initially multiple independent emergences of artemisinin resistance occurred,14,27,28 but hard selective sweeps by presumably fitter parasites have gradually replaced the first soft selective sweeps.13,24 The reduced parasite killing associated with artemisinin resistance increased the selective pressure on their ACT partner drugs, and resistance to them followed. ACT treatment efficacy has declined correspondingly.14,25

In terms of malaria epidemiology, the Greater Mekong subregion can be divided into two discrete transmission regions: the eastern region, comprising Cambodia and adjacent southern Vietnam, southern Laos, and northeastern Thailand, and the western region, comprising Myanmar and adjacent western Thailand, with borders with Yunnan province, China, northeast India, and Bangladesh. The area between these two regions, comprising northern Laos and the main body of Thailand, is largely malaria free. In the eastern region, except for Laos, dihydroartemisinin–piperaquine has been used extensively for many years. A single long haplotype pfkelch Cys580Tyr mutant parasite lineage (PfPailin) has now spread across the eastern Greater Mekong subregion and has also acquired piperaquine resistance (associated with pfplasmepsin2 amplification and pfcrn mutations).13,24

Figure 5: Frequency distributions of pfmdr1 gene amplification in four countries of the Greater Mekong subregion, 2007–18

CNV = copy number variation.

dihydroartemisinin–piperaquine in the sites in Myanmar and Cambodia. There was also no evidence for selection of pfcrn mutations associated with piperaquine resistance; none were found in Kayin State, Myanmar, and pfcrn Gly353Val was found in seven isolates before and seven after mass treatment in Cambodia (with a single haplotype of CVIET at residues 72–76; figure 6; appendix p 15).10–13,27 There was also no evidence for further selection of pfkelch mutations after mass treatment.

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Figure 6: Frequency distributions of pfplasmspin2 gene amplification, pfcrtp, and pfkelch gene mutations in Plasmodium falciparum isolates from submicroscopic parasitaemias in eastern Myanmar and western Cambodia, before (n=142) and after (n=38) mass drug administration with dihydroartemisinin and piperaquine.

Dihydroartemisinin–piperaquine was initially highly effective and became first-line treatment in Vietnam, Cambodia, and Thailand, but as the multidrug-resistant PfPailin lineage came to dominate in the eastern Greater Mekong subregion, high rates of treatment failure occurred. Treatment failure increased with the acquisition of mutations in pfcrtp,11 which reduced piperaquine susceptibility,11,16 and pfplasmspin amplification, which is associated with reduced piperaquine susceptibility. These new pfcrtp mutations are downstream of the 4-aminoquinoline resistance locus (positions 72 to 76), which is mutated as the CVIET haplotype in nearly all P falciparum parasites from the region.

The majority of falciparum malaria in Thailand used to occur in areas adjacent to Myanmar, with the highest case numbers in Tak province.29 Since provision of support for village malaria workers and introduction of concerted malaria elimination activities (including mass drug administrations) in adjacent Kayin state in eastern Myanmar,17,30–32 the incidence and prevalence of falciparum malaria on both sides of the border has dramatically declined.29 Before these large-scale interventions, a single pfkelch CysS80Tyr haplotype predominated.27 Furthermore, despite intense use of dihydroartemisinin–piperaquine in treatment and in mass treatments in Kayin state,28,14 there is no evidence for emerging piperaquine resistance there.33 No pfplasmspin2 amplification and no pfcrtp mutations associated with piperaquine resistance were identified in this region and, although low prevalences of pfplasmspin2 amplification were found elsewhere in Myanmar, there was no evidence for selection. There was also no evidence for the spread of drug-resistant P falciparum from the east.
to the west of the Greater Mekong subregion. There is therefore still a window of opportunity to continue the elimination of multi-drug resistant malaria in the western Greater Mekong subregion using dihydroartemisinin–piperquine in targeted mass treatments, as proved successful in eastern Kayin state. In Myanmar, a *P falciparum* lineage bearing the *pfkelch* Phe446Ile mutation,\(^1^,^6^,^1^8^) recognised first on the northern border with Yunnan, China, has now spread extensively across the country. In northern Myanmar, Phe446Ile comprises the majority of *pfkelch* propeller mutants. This evolving pattern, in which a single parasite lineage dominates, is similar to that observed earlier in the eastern Greater Mekong subregion with the Cys580Tyr mutation,\(^3^,^1^8^) which has not dominated in artemisinin resistance (in terms of slowing of parasite multiplication in or around the elimination areas).\(^3^3^)

The selection and sub-division of parasites in or around the elimination areas shows no evidence that mass treatments selected for artemisinin or piperquine resistance. This finding supports the use of targeted mass treatment in a low transmission setting to reduce the burden of malaria in foci of high transmission, thereby reducing the risk of resistance emerging.\(^3^)

*pfdmr1* amplification occurs readily within malaria infections and was largely responsible for the rapid reduction in mefloquine susceptibility on the eastern and western borders of Thailand in the early 1990s before ACTs were introduced.\(^3^) Despite this reduction in mefloquine susceptibility, artesunate–mefloquine proved highly effective in the same areas and gave sustained efficacy for more than 15 years before failure rates increased sharply again in 2011.\(^1^4^) The reduction in artesunate-mefloquine efficacy resulted from the combination of artemisinin resistance (associated with *pfkelch* mutations) and re-emerging mefloquine resistance (associated with *pfdmr1* amplification).\(^7^) Our survey shows that the prevalence of *pfdmr1* amplification has declined again on both the eastern and western borders of Thailand and adjacent countries after artesunate–mefloquine was replaced as first-line treatment. This finding is somewhat reassuring for artemether-lumefantrine, the first-line treatment in Myanmar\(^9^) and Laos, although how much longer efficacy will be preserved in the face of artemisinin resistance is uncertain. Concern over the longevity of current ACTs has led to proposals for use of triple ACTs to protect the more slowly eliminated antimalarial drugs and prolong their utility.\(^1^1^)

The main limitation of this study is that sampling was usually associated with studied interventions, so it was focused in areas already known to have a higher incidence of malaria than elsewhere. Although the tested samples were obtained from multiple sites across the region this was not planned as a geographic survey and, as with many other observational molecular epidemiology studies, might therefore give a biased picture of genotype distributions. Surveillance by genotyping routine *P falciparum* positive rapid diagnostic test samples from village health workers would clarify the geographic distribution and monitor the spread of resistance. The marked reduction in the incidence of falciparum malaria in areas where targeted malaria elimination activities have been done means that few parasite isolates remain to provide accurate estimates of the prevalence of resistance markers after the interventions. But the low number is itself a testament to success. Nevertheless, the overall size, duration, and geographic extent of this investigation does allow characterisation of the emergence and spread of antimalarial drug resistance in the region, and it gives confidence in concluding that dihydroartemisinin–piperquine mass treatments have not selected resistance further.

The WHO strategy for malaria elimination in the Greater Mekong subregion (2015–30)\(^3^5^) set the following targets: by 2020 or earlier transmission of *P falciparum* malaria to be interrupted in all areas of multidrug resistance, including ACT resistance; by 2020 *P falciparum* malaria to be eliminated in Cambodia; and by 2025 *P falciparum* malaria to be eliminated in all Greater Mekong countries. These targets will clearly not be met. The selection and subsequent spread of fit multidrug-resistant parasites across the large landmasses of southeast Asia has been repeatedly highlighted as a major threat to global malaria control. The solution is to eliminate falciparum malaria in the Greater Mekong subregion before it spreads further. There is still time for radical action before drug resistance prevents it.

**Contributors**

MI, MD, SaP, FMS, FHN, and NJW contributed to study design. MD, KMT, AMT, APP, RV, CP, AS, NK, RS, ST, NS, KS, RTH, YH, KL, AAK, TMH, RWvdP, MM, TP, TP, RF, RT, LS, CN, DL, XHSC, HR, RL, CH, DPK, OM, MM, RT, MD, FHN, FMS, AMD, and NJW organised or conducted clinical and epidemiological investigations. MI, KaS, CK, SS, and JD did the molecular genotyping. MI, SiP, NPD, AMD, FMS, FHN, www.thelancet.com/infection Published online July 14 2020 https://doi.org/10.1016/S1473-3099(20)30228-0 9
and NJW analysed the data. MI and NJW prepared the report. All authors read and approved the final manuscript.

Declaration of interests
We declare no competing interests.

Data sharing
The data are available upon request to the Mahidol–Oxford Tropical Medicine Research Unit Data Access Committee for researchers and access follows the Mahidol–Oxford Tropical Medicine Research Unit data access policy. Queries and applications for datasets should be directed to Rita Chanviriyavuth (Mahidol–Oxford Tropical Medicine Research Unit; rita@tropmedres.ac).

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