do not fully respond to treatments with dihydroartemisinin–piperaquine should be recommended. In the long term, we must optimise current artemisinin-based combination therapies and investigate potentially increasing the duration of artemisinin administration. Focusing on triple combination therapies could become a distraction and miss a crucial opportunity to achieve urgent elimination of malaria parasites before new complications arise.

SK is a member of the WHO Malaria Treatment Guidelines Group. This group produces global guidance on the treatment of malaria and this includes decisions on artemisinin combination therapies. The views expressed here are personal opinions and do not represent the recommendations of WHO. All other authors declare no competing interests.

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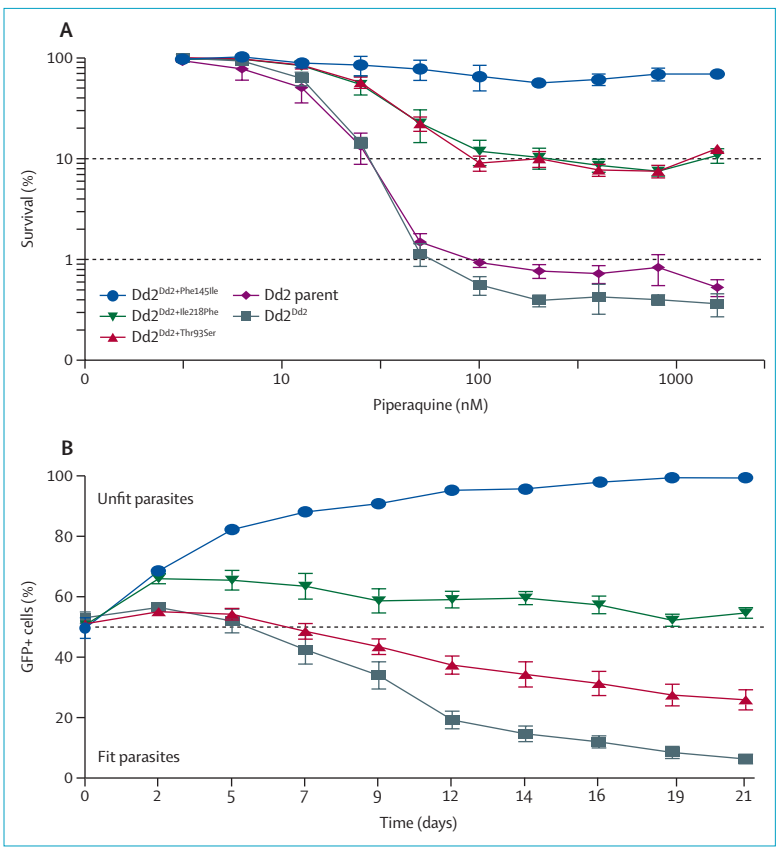

Figure: **Piperaquine survival and fitness of *pfcrt*-edited lines**
(A) Survival of *pfcrt*-modified parasite lines cultured with various concentrations of piperaquine (0–6 h rings, treated for 48 h). The 10% cutoff represents a standard working threshold for piperaquine resistance at 200 nM piperaquine. Data are mean with SEM for three to four independent assays done in duplicate. (B) In-vitro growth of the indicated parasite lines in competition with a GFP-positive reporter line. Data are mean with SEM for two independent experiments done in triplicate. Methods are provided in the appendix. GFP=green fluorescent protein.

See Online for appendix

Plasmodium falciparum resistance to piperaquine driven by PfCRT

The Articles by William Hamilton and colleagues[1] and Rob van der Pluijm and colleagues[2] illustrate the plummeting clinical efficacy of dihydroartemisinin–piperaquine as a first-line treatment for *Plasmodium falciparum* malaria in southeast Asia. These authors also report a rapid regional spread of clonal parasite lineages harbouring novel variants of the *P falciparum* chloroquine resistance transporter PfCRT (emerging on the chloroquine-resistant Dd2 isoform). These lineages exclusively harboured the Cys580Tyr mutation in the *K13* gene that is associated with decreased artemisinin efficacy. These studies raise important questions about whether these novel PfCRT variants cause piperaquine resistance, how they effect other antimalarials, and whether changes in prevalence over time reflect differences in parasite fitness.

Here, we show that the now predominant PfCRT Thr93Ser and Ile218Phe mutations, which of all mutations have expanded the most rapidly in the past 5 years,[1,2] confer piperaquine resistance when individually edited into the *pfcrt* locus of Dd2 parasites. Assays with cultured intra-erythrocytic parasites show 8–13% survival at piperaquine concentrations ranging from 200 nM to 1600 nM (figure; appendix p 5). The Phe145Ile mutation was highly piperaquine-resistant, with 57–69% survival at these elevated concentrations, consistent with previous findings.[3] Parental or control Dd2 parasites with an edited *pfcrt* showed less than 1% survival, reflecting background

rates. Quantitative PCR assays showed a single copy of plasmepsin II in all edited parasites (data not shown), indicating that amplification of this initial resistance marker[4,5] was not required for piperaquine resistance.

Whole-genome sequence data of 84 Cambodian isolates support the expansion of Thr93Ser and Ile218Phe between 2013 and 2016, overtaking the prevalence of Phe145Ile (appendix p 4). Recent years showed a reduced percentage of parasites harbouring four or more copies of plasmepsins II and III, although parasites with two to three copies remained the majority (appendix p 4). *pfmdr1* amplification, a marker of reduced susceptibility to lumefantrine and mefloquine, became less common over time (appendix p 4). Survival rates of piperaquine-treated cultured parasites increased over the years (appendix p 4), mirroring increasing dihydroartemisinin–piperaquine clinical failure rates. Emerging PfCRT mutations also increased *P falciparum* susceptibility to chloroquine, amodiaquine, quinine, pyronaridine, and ferroquine, with the Phe145Ile mutation causing the greatest sensitisation (appendix pp 6, 7). Dihydroartemisinin, lumefantrine, and mefloquine were unchanged. These data highlight the broad effect of PfCRT mutations on multiple antimalarials.

To test for differences in parasite fitness between mutants, we used a competitive growth rate assay in which each parasite line was individually cocultured with an isogenic green fluorescent protein (GFP)-positive Dd2 line. Parasites expressing the Thr93Ser allele showed a negligible fitness cost compared with control Dd2 *pfcrt*-edited parasites, with both lines out-proliferating GFP-positive Dd2 parasites (figure). The Ile218Phe mutation showed a mild growth attenuation. Phe145Ile parasites showed a substantial fitness cost, potentially explaining why this allele is ceding ground to the less resistant but fitter Thr93Ser and Ile218Phe mutations.

The data support a key role for PfCRT mutations in driving the recent expansion of highly piperaquine-resistant parasites in southeast Asia and highlight the need for vigilance in screening for novel PfCRT mutations in other malaria-endemic regions, notably in Africa or South America where piperaquine use has been increasing.

We declare no competing interests.

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No measles cases during the 2019 Hajj

The 2019 Hajj took place amid a global surge in measles cases, including travel-associated infections.[1] Eduard Massad and colleagues[2] estimated that at least 110 measles importations would occur during the 2019 Hajj, mainly from six countries (Yemen, India, Nigeria, Indonesia, Pakistan, and Sudan). They warned that these imported cases could trigger a significant outbreak towards the end of the Hajj, leading to rapid international dissemination when pilgrims return to their home countries. Others voiced similar concerns regarding the importation and the potential globalisation post-Hajj of measles via returning pilgrims.[3]

Saudi Arabia implements numerous measures to prevent communicable diseases during the Hajj, including vaccination requirements, health awareness campaigns before and during the event, and public health interventions at points of entry. Additionally, during the Hajj, Saudi health authorities activate an enhanced indicator-based notifiable diseases surveillance system to ensure timely detection and prompt response to infectious disease events.[4] The 2019 Hajj also saw the first application of the Hajj early warning system (HEWS), a syndromic and event-based surveillance system that complements the existing surveillance tools at the pilgrimage.[4]

The 2019 Hajj was attended by 2 489 406 pilgrims, 74·5% of whom were international. Almost 746 000 people originated from the six counties identified as posing the greatest risk for measles importation.[2,3] Measles was identified as a high-risk event in the 2019 Hajj strategic health risk assessment conducted by the Saudi health authorities, and was an essential syndrome of the HEWS. No cases of measles or outbreaks of infectious diseases were reported during the 2019 Hajj, from early July when pilgrims started arriving in the country until the pilgrimage officially ended in mid-August.

Although this is reassuring, given the incubation period of measles, as pilgrims return to their home countries the possibility of post-Hajj spread remains. We echo others in calling for public health measures to