Simple, rapid test for drug-resistant malaria developed

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For the first time, scientists have developed a novel and rapid way to test whether the most common and lethal form of malaria is resistant to potent artemisinin drugs.

"In the race against time to stop the spread of artemisinin-resistant malaria, new diagnostic tools are urgently needed to identify and track resistant parasites. These simple in-vitro and ex-vivo ring-stage survival assays (RSAs) can clearly identify artemisinin-resistant, slow-clearing *Plasmodium falciparum* parasites in people with malaria, and can deliver results much faster than the current clinical approach used to monitor response to drugs in patients", explains study leader Didier Menard from the Institut Pasteur du Cambodge in Cambodia, in *The Lancet Infectious Diseases*.

More than 200 million people are infected with the malaria parasite *P falciparum*, which kills between 655 000 and 1.2 million people every year. Antimalarial control efforts are mainly dependent on artemisinin-based combination treatments (ACTs), having replaced older drugs that the malaria parasite developed resistance to. Should these regimens fail, no other drugs are ready for widespread use, and eliminating malaria in the near future might then prove impossible.

Until now, standard laboratory artemisinin susceptibility tests have been unable to distinguish the slow parasite clearance seen in people who are infected with drug-resistant parasites from normal, fast parasite clearance.

In this study, a team of researchers from the Cambodian National Malaria Centre, the Institut Pasteur, Phnom Penh and Paris, and the US National Institutes of Health set out to test whether a novel in-vitro RSA could distinguish slow-clearing from fast-clearing parasites, investigate the in-vitro response to dihydroartemisinin (DHA; the active metabolite of all artemisinins) of three different blood stages of parasites (early rings, late rings and trophozoites), and assess whether an ex-vivo RSA might detect artemisinin-resistant *P falciparum* infections.

Parasites from Pursat province, an area of artemisinin resistance in western Cambodia, and Preah Vihear and Ratanakiri, regions of artemisinin sensitivity in northern and eastern Cambodia were exposed to a clinically-relevant pulse of DHA (six-hour pulse of 700 nM) and their survival measured 72 hours later.

Although late rings and trophozoites from slow-clearing and fast-clearing infections showed no difference in their susceptibility to DHA, early rings (0–3h post invasion) from slow-clearing parasites survived DHA significantly better than those from fast-clearing parasites.

"We were able to clearly see the difference in the clinical response to artemisinins between people infected with parasites that were drug-resistant or drug-susceptible in vitro", says Menard. "Our observations confirm that artemisinin resistance is associated with the very early stages of parasite development in the blood."

In the ex-vivo RSA, parasite survival rate also significantly correlated with parasite clearance half-life (the time it takes for the drug to reduce the number of parasites in the blood by half), and the test accurately detected slow-clearing infections in the Preah Vihear and Ratanakiri provinces of Cambodia where they have not yet been described.

According to study co-leader Rick M Fairhurst from the US National Institutes of Health, "The in-vitro RSA can be used to understand molecular mechanisms of artemisinin resistance, to investigate the mode of action of artemisinins, and to screen and identify next-generation antimalarial drugs that can effectively kill artemisinin-resistant parasite strains. On the other hand, the ex-vivo RSA can be readily implemented in field-based

settings to monitor the worsening of artemisinin resistance in Cambodia where it is highly prevalent and to map its spread to other regions of southeast Asia. Also, this simple test can be easily established at sentinel sites in sub-Saharan Africa, where the arrival or evolution of artemisinin-resistant *P falciparum* is expected to be particularly devastating."

Writing in a linked Comment, Carol Hopkins Sibley from the WorldWide Antimalarial Resistance Network (WWARN) says, "These assays will allow the rapid validation of candidate molecular markers by directly testing the correlation of proposed markers with the output from their survival assay. The in-vitro test will also provide a platform for understanding the mechanism of the reduced artemisinin response. With these simple methods in place, rapid tracking of the geographical and temporal changes in artemisinin resistance will be feasible in many sites. This far more comprehensive information will allow policy makers to design effective responses to the threat of artemisinin failure, and prolong the useful therapeutic life of ACTs."

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