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Combating drug resistance in malaria

Concerns that antimalarial treatments are losing their effectiveness against *Plasmodium* parasites have set scientists scrambling to slow the spread of resistance and develop alternative drugs. **By T. V. Padma**

For people living in parts of the world where malaria is endemic, losing loved ones to the mosquito-borne disease has long been a fact of life. "As a young girl growing up in Africa, the disease claimed lives in so many families, including my siblings [and] neighbours," says Corine Karema, a Rwandan epidemiologist and interim chief executive at the RBM Partnership to End Malaria (formerly known as Roll Back Malaria). "That was normal," she says. "It was accepted."

Over the past few decades, however, the image of malaria as an inevitability has begun to change. In 2000, the annual incidence of malaria in Africa was estimated at 373 cases per 1,000 people living in areas where transmission occurs; in 2021, the figure was 229 cases per 1,000 people. Deaths owing to malaria are falling even faster. In Rwanda, where Karema led a national malaria-control programme between 2006 and 2016, the malaria mortality rate has dropped by nearly 70% since 2000. "Losing family members due to malaria is no longer a norm," says Karema.

Africa's progress in the control of malaria rests on several strategies. There has been a marked increase in the use of prevention tools, such as mosquito nets treated with insecticides. Around two-thirds of people who are at risk of malaria in sub-Saharan Africa had access to such nets in 2015, compared with just 7% a decade earlier. The fight is also heavily reliant on antimalarial drugs that target the *Plasmodium* parasites carried by mosquitoes.

The most notable are treatment regimens that involve a drug based on artemisinin. These have been used to shorten hospital stays, reduce mortality and even help to bring down transmission. But a problem is emerging: the parasites are increasingly resistant to artemisinin-based treatments. Resistant parasites were first reported around 15 years ago in southeast Asia. Later, they were detected in Africa — initially in Rwanda in 2014 (ref. 1).

With no alternative drugs with similar safety and efficacy available, researchers are warning of a major health crisis if resistance becomes widespread in Africa. By one estimate, if the failure rate of artemisinin-based treatments increased modestly from 5% to 30%, the mortality rate would rise by around 20% (ref. 2).

Researchers, physicians and public-health authorities are working to combat this threat.

Part of the plan to prevent resistance — or at least delay it — is to use available drugs in smarter ways. There are also growing calls for greater surveillance of drug resistance, as well as better use of prevention strategies that might reduce reliance on drugs. Some researchers, meanwhile, are developing drugs that could be used in place of artemisinin-based therapies where resistance is high — although overcoming resistance entirely could prove impossible.

A growing problem

Of the 150 or so species of *Plasmodium* parasites, just 5 are known to cause malaria in people. Most cases are caused by either *Plasmodium falciparum* or *Plasmodium vivax*, and treatment depends on which species a person is infected with.

For *P. vivax*, the first-line treatment is typically chloroquine. This is a synthetic version of quinine, which is derived from the bark of cinchona trees. Developed in 1934, chloroquine was initially also used against *P. falciparum*, the deadliest malaria parasite and the most prevalent in Africa. But over time, resistance emerged. *P. falciparum* mutated in a way that helped it to clear the drug from its system, reducing its effectiveness and forcing a change of tack. *P. falciparum* infection is now treated mainly with artemisinin, a compound isolated from the plant sweet wormwood (*Artemisia annua*).

Artemisinin's antimalarial properties are thought to stem from a structural feature of the molecule called a peroxide bridge. In the presence of iron, which the parasites are rich in owing to their taste for human haemoglobin, this structure breaks down and releases highly reactive free radicals that can damage key parasite proteins. The result is that artemisinin and its derivatives, including artesunate, artemether and dihydroartemisinin, swiftly reduce the number of parasites in a person's blood. Artemisinin is about 90% effective in curing uncomplicated malaria (a form characterized by fever, headaches and muscle pain).

Although artemisinin is highly potent and kills most of the parasites, it is rapidly cleared by the body. That's why artemisinin is now typically given in combination with other drugs that are structurally similar to chloroquine, such as mefloquine and piperaquine. These partner drugs last longer, and should kill any parasites that escape the artemisinin assault.

The World Health Organization (WHO) recommends that artemisinin-based combination therapies (ACTs) should be the first- and second-line treatment for uncomplicated *P. falciparum* malaria, as well as for chloroquine-resistant *P. vivax* malaria. But increasing reports of resistance to artemisinin among *P. falciparum* parasites, as well as to the partner drugs that are given alongside it, could threaten the effectiveness of this strategy.

Researchers have linked artemisinin resistance to mutations in the *PfK13* gene. These mutations disrupt the parasite's ability to breakdown haemoglobin, reducing iron levels in the parasite. Because iron seems to be involved in activating artemisinin, the lower levels probably reduce the drug's effectiveness[3]. So far, around 20 mutations in *PfK13* have been linked to artemisinin resistance, but this might not be the whole story. "It is possible that other mechanisms, which have not yet been identified are playing a role — or will do so in future," says Philippe Guérin, an epidemiologist at the Infectious Diseases Data Observatory in Oxford, UK.

"Mutations that limit the effectiveness of artemisinin are now being reported in sub-Saharan Africa."

The clinical consequence of artemisinin resistance is that instead of parasite clearance taking three days it can, in some cases, take up to a month, says Tim Wells, chief scientific officer at Medicines for Malaria Venture, a non-profit organization in Geneva, Switzerland.

For now, Wells says, the impact on individuals seems to be minor. But more ominous consequences could lie ahead because the longer clearance time gives the parasites more opportunity to develop resistance to the partner drug in ACT. "If the partner drug fails, then the combination does not work," says Wells.

Resistance to artemisinin was described in Cambodia in the late 2000s[4]. This was followed by reports across the Greater Mekong area, including in Thailand, Vietnam, Laos and south China. Over the past seven or eight years, resistance has started to emerge in Africa and parts of South America.

"Mutations that limit the effectiveness of artemisinin [...] are now being reported in sub-Saharan Africa, posing a serious threat to malaria-endemic countries that rely on ACTs to treat malaria," says disease modeller Philip Welkhoff, who directs the malaria programme at the Bill & Melinda Gates Foundation in Seattle, Washington. It is likely that these mutations are emerging independently of those in Asia, rather than spreading from one continent to another. Most of the mutations found in sub-Saharan Africa are different from those in southeast Asia.

In Rwanda, parasites with one *PfK13* mutation, which is associated with delayed clearance, were found in the blood of one in five people taking part in an antimalarial drug trial in the capital Kigali between 2018 and 2019 (ref. 5). Uganda and Eritrea have also reported mutations linked to resistance. Fortunately, the partner drug lumefantrine is still effective, and failure rates of treatment in Rwanda and Uganda have so far remained below 10%.

Beating resistance

To maintain the efficacy of antimalarial drugs for as long as possible, therapies should be used in ways that minimize the risk of resistance developing. "The real question is how can we delay the emergence of resistance?" says Guérin.

One strategy is to avoid administering artemisinin on its own. "It is likely that the massive use of artemisinin derivatives in single drug-based treatment, prior to their introduction in combination, has contributed to the emergence of resistance," says Guérin. In 2007, the WHO called for oral artemisinin-based monotherapies to be removed from the market, but some governments, manufacturers and sellers have defied this resolution. A study in Nigeria, for example, showed that in 2015, 84% of pharmacies offered artemisinin monotherapies despite a national ban[6]. The use of *Artemisia* teas as herbal remedies has also been identified as a potential source of resistance[7].

Malaria prevention strategies, such as vaccination or the use of insecticide-treated bed nets, can also help to forestall resistance — fewer people who require treatment means less selection pressure on parasites. Worryingly, however, mosquitoes are increasingly resistant to pyrethroids, the main insecticides used in antimalarial bed nets and indoor spraying.

The emergence of resistance can also be slowed by rotating the drugs in use. In Cambodia, health workers swapped artesunate and mefloquine for dihydroartemisinin and piperaquine, and then changed back again. But this is logistically challenging. It takes time to procure a new supply, get the drugs to where they are needed, withdraw the old ones and train health-care providers to administer the new treatment. And while this is happening, resistance will continue to spread. "Each time an ACT fails, patients are still treated with this suboptimal ACT until a new ACT is introduced," says Chanaki Amaratunga, an immunologist at the Mahidol Oxford Tropical Medicine Research Unit, Bangkok. "During the time it takes to make this change, there is opportunity for the spread of multidrug

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A person takes part in research in Cambodia, where artemisinin resistance has been reported.

resistant parasites," she says.

Amaratunga's team is exploring triple combination therapies, which combine an artemisinin derivative with two partner drugs, on the assumption that it's highly unlikely that a parasite will become resistant to all three at once. "The partner drugs provide mutual protection to each other," Amaratunga says. "We have shown that these triple ACTs are safe, well tolerated and highly efficacious." Her team is now assessing the feasibility of introducing triple ACTs as first-line treatments for malaria.

Some researchers are now working out how to better detect and track resistance. "In order to react to antimalarial resistance and take preventive measures, we need to know where it is prevalent," says Guérin.

Resistance to antimalarial drugs can be monitored in a few different ways, Guérin explains. One method is to track how people respond to the treatments that they are prescribed. However, this is logistically challenging and costly, and is vulnerable to confounding factors such as adherence to medication, nutrition status, pregnancy and acquired immunity. Another option is to evaluate parasites in the laboratory — either to assess their susceptibility to the drugs, or to search for molecular markers of drug resistance. Such markers have been validated for several artemisinin derivatives and for some partner drugs, including chloroquine, sulfadoxine and pyrimethamine, piperaquine, amodiaquine and mefloquine. "Their prevalence in a parasite population is often a good indicator of the level of clinical resistance," Guérin says. But testing of this kind requires substantial lab infrastructure and staff with appropriate training. The low- and middle-income countries where malaria is endemic often lack both.

Search for alternatives

With artemisinin resistance rising, scientists are also working on alternative drugs. "Targeting of non-artemisinin-based-mechanisms using novel drugs is already a focus of considerable research worldwide," says Amit Prakash Sharma, a parasitologist at the International Centre for Genetic Engineering and Biotechnology in New Delhi.

Some of those being tested by research groups, including Sharma's, target a key enzyme involved in the parasites' protein synthesis, called aminoacyl transfer RNA synthetase. But other mechanisms are also involved, he adds. For example, non-artemisinin-based drugs can target signalling pathways that are crucial to the invasion of the host's red blood cells by parasites, or processes involved in making key proteins that are essential to the parasites' survival.

The most advanced treatment in clinical development is a combination of ganaplacide and lumefantrine. This targets the malaria parasite when it grows and multiplies in a person's liver, as well as later in its life cycle when it develops inside red blood cells.

Phase II trials, in which the combination was given daily for three days, suggest it is safe and effective against both *P. falciparum* and *P. vivax* — including artemisninin-resistant parasites. Efficacy might meet the high bar set by ACTs, says Guérin. A phase III trial began in November 2022, as a partnership between Medicines for Malaria Venture and Swiss pharmaceutical giant Novartis. Results are expected by 2025.

Despite the flurry of research, concerns remain that some of the non-artemisinin drugs in development might not be able to overcome existing resistance. Most of those in late development will be combined with partner drugs that are either already used in ACTs or are from the same chemical families, says Guérin. "If those partner drugs develop resistance, or have already developed resistance, it is likely that the new compounds will not be protected, and resistance may emerge rapidly."

The deployment of ACTs has made it clear that there is a strong basis for this concern. In India, for example, a combination of artesunate and partner drug sulfadoxine and pyrimethamine was made the first-line drug for use against uncomplicated *P. falciparum* infection in 2010. In three years, Sharma says, resistance to that combination had emerged in northeast India, requiring the combination to be changed to artemether and lumefantrine.

Even if treatments are entirely new, these too are likely to come up against similar issues of resistance eventually. Parasites will evolve to counter non-artemisinin-based treatments over time, says Sharma. "This has happened repeatedly in the past."

The speed at which this happens, however, could be influenced considerably by the drugs' design. Some mechanisms of action will be easier for parasites to resist than others. Typically, the more important the function is to *Plasmodium*, the less likely it is that the parasite will develop resistance to the drug that interferes with it. "It is feasible to target essential biological processes within the malaria parasites using multiple drugs so that the development of resistance is either severely delayed or reduced to a very small possibility," says Sharma.

Until new treatments arrive, the focus for those fighting malaria is to ensure that the armoury available can be used for as long as possible. Drugs and insecticidal nets have contributed extensively to malaria reduction, Sharma says. It seems inevitable that emerging resistance to both will harm this progress. "The race," Sharma says, "is to eliminate malaria from as many regions as possible before the onset of drug and insecticide resistance."

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