Malaria – update on antimalarial resistance and treatment approaches

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Funding: SY is supported by a grant from the UK Department for International Development for the Tracking Resistance to Artemisinins Collaboration 2 (TRAC2) research programme

Conflicts of interest: None

Keywords: Malaria, drug resistance, *P. falciparum*, Artemisinin-based combination therapy

Abbreviated title: Malaria – update on drug resistance and treatment

Running head title: Malaria – drug resistance and treatment

Introduction

Malaria continues to be a major cause of childhood mortality and was responsible for an estimated 303,000 (165,000-450,000) deaths in children aged under 5 years in 2015. However, this represents a 60% reduction in mortality since 2000 [1], one of biggest successes in terms of the Millennium Development Goals. Central to this achievement was the widespread deployment of effective tools for prevention and treatment, including insecticide treated nets and Artemisinin-based Combination Therapies (ACTs). The recent emergence and spread of *Plasmodium falciparum* (Pf) parasites resistant to ACTs, and mosquitoes resistant to the pyrethroids, the most commonly used insecticide, threaten to reverse these gains, and the hopes of eliminating malaria. This review provides an update on antimalarial resistance and approaches to treatment.

Types of malaria

Nearly all malaria related deaths are due to Pf, which is also the most drug-resistant of the five species of *Plasmodium* to infect humans, and is the main focus of this review. *P. vivax* (Pv) which has a wider geographical spread and can cause severe disease, is also becoming increasingly resistant to chloroquine, and was the subject of a recent PIDJ review [2]. Zoonotic infections with *P. knowlesi,* which usually infects long-tailed macaques, are increasingly recognised as an important cause of human malaria in parts of Southeast Asia, especially Malaysia where it is responsible for over 70% of malaria cases, of which 10% are severe. It is most effectively treated with ACTs. *P. malariae* and *P. ovale* remain sensitive to chloroquine, but can also be treated effectively with ACTs. Although the cost of ACTs used to be higher than other antimalarials, their cost has fallen significantly and a number of countries now have simplified treatment guidelines which recommend ACTs for all species of malaria.

Advances in malaria diagnostics

Previously microscopy was the mainstay for parasitological diagnosis. However, it requires skilled microscopists, functioning microscopes and a reliable supply of reagents. Therefore antimalarials were often taken presumptively, without parasitological confirmation, giving rise to concerns of under-treatment of patients with malaria, and overuse of antimalarials in patients without, and the associated risks in terms of drug resistance.

The advent of malaria rapid diagnostic tests (mRDTs) in the last 10 years have transformed the diagnostic landscape. Quality-assured mRDTs are sensitive and specific, provide a result within 20 minutes, are affordable (~€0.50 per test) and easy to use. Tests detect either Histidine Rich Protein 2 (HRP2), which is a Pf-specific antigen, and/or the pan-species antigen *Plasmodium* Lactate Dehydrogenase and have a similar sensitivity to good microscopy. Over 200 million mRDTs were distributed by national malaria programmes in 2015, largely enabled by donor support. Microscopy still has an important role, in terms of quantification of parasite density, staging and treatment follow-up. Of note HRP2 tests can remain positive several weeks after treatment so are not useful for follow-up. Secondly, parasites with HRP2 deletions have been detected, allowing them to evade detection by mRDT. Although prevalence rates of up to 40% have been reported from the Amazonian basin in Peru, they are much rarer elsewhere and currently not thought to be a major cause of false negative results. Although more sensitive diagnostics are available which are able to detect parasite densities more than 10-fold lower then microscopy and standard mRDTs, their role is currently limited to research and surveillance. These include PCR, Loop-mediated isothermal amplification (LAMP) and "Ultra-sensitive" HRP2 mRDTs,

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Antimalarial treatment

Artemisinin-based combination therapies are the mainstay of treatment for Pf malaria. Artemisinin derivatives, or "Qinghaosu", had been used to treat fever in China more than two thousand years ago and were re-discovered by Chinese scientists during the American-Vietnam war. They act on a broader range of parasite blood stages than any other antimalarial and are the most rapid acting, reducing (sensitive) parasite loads by an order of 10^5 fold every 48 hours. For uncomplicated Pf, they should always be given in combination with another effective drug with a different mechanism of action, ideally as a fixed dose combination. This is for two reasons: firstly, on their own they need to be taken for at least 7 days which is poorly adhered to, and secondly to minimise the development of parasite resistance. The partner drugs currently used in ACTs include: lumefantrine, amodiaquine, piperaquine, mefloquine, pyronaridine and sulfadoxine-pyrimethamine (SP). As described later, in the Greater Mekong Sub-region (GMS), parasite have developed resistance to all partner drugs. In Sub-Saharan Africa resistance to SP is widespread but the other partner drugs remain effective. The use of SP should be avoided in individuals who have HIV/AIDS, and the use amodiaquine should also be avoided if they are being treated with efavirenz or zidovudine due to the risk of exacerbation of hepatotoxicity and neutropenia respectively [3].

For severe malaria, initial treatment should be with intravenous artesunate for at least 24 hours followed by a full course of oral ACT. Children weighing < 20 kg should receive a higher dose of artesunate (3 mg/kg per dose) than larger children and adults (2.4 mg/kg per dose) [3]. The largest randomized clinical trials ever conducted on severe falciparum malaria showed a substantial reduction of mortality with parenteral artesunate compared with parenteral quinine. It is also safer, better tolerated, easier to administer (including once per day administration), and cost-effective [4, 5]. Artesunate suppositories are also now available, enabling pre-referral administration to children

with severe malaria in remote settings, a potentially life-saving innovation. Quinine remains a useful second-line drug for severe malaria.

Supportive and adjunct therapy

Children with complicated malaria require close monitoring of vital signs, fluid balance, glucose, biochemical and haematological markers. These should be used to guide resuscitation with fluids, glucose, and blood, while avoiding rapid bolus infusions. Co-infection with bacteria is not uncommon and all children with severe malaria should also receive intravenous antibiotics, pending blood culture results. Haemofiltration should be considered early in children with renal dysfunction. Although there is anecdotal experience of exchange transfusions, there is insufficient evidence to make any practical recommendations. Similarly adjunctive therapies including immune modulators (high-dose corticosteroids, anti-TNF agents, cyclosporin, hyperimmune serum) and anticoagulants have been evaluated with varying results in terms of effectiveness and safety.

Antimalarial resistance

Treatment failure and parasite clearance times

Clinically, drug resistance first manifests as the slower clearance of parasite from the blood stream and longer time for patients to defervesce [6, 7]. As resistance worsens, less sensitive parasites survive and multiply resulting in recrudescent parasitaemia and treatment failure. The interval between initial treatment to recrudescence depends on the level of resistance, patient immunity and the pharmacokinetic-pharmacodynamic relationship. Drugs with long half-life such as mefloquine and piperaquine, continue to exert some inhibitory effect on partially resistant parasites for weeks, so infections may not recrudesce for several weeks. For drugs with short half lives, and with parasites which are more resistant (and therefore able to grow in the presence of drugs), the

interval to recrudescence can be a matter of days and in extreme cases there will be no initial clearance of parasites.

It is worth noting that resistant parasites are not the only cause of recrudescent infections. Recrudescence can occur due to sub-therapeutic dosing which in turn can be due to an inadequate dose being prescribed, poor patient adherence to a correctly prescribed regime, poor absorption (particularly for lumefantrine which needs to be taken with fatty food)– or poor quality drugs. The latter is extremely common in malaria-endemic countries where studies have shown the prevalence of poor quality drugs (defined as <85% of stated active ingredient) to be as high as 31% [8]. In addition to recrudescence, recurrent infections can also be due re-infection or relapse which refers to the recurrence of blood-stage infections due activation of hypnozoites in *P. vivax* and *P. ovale* infections.

Monitoring of antimalarial resistance

In malaria-endemic countries routine monitoring of antimalarial drug efficacy is carried out at sentinel sites by national malaria control programmes using a standardised WHO protocol. Treatment response is defined as the absence of parasitaemia at follow-up, on day 28 or 42. WHO recommends that when a 10% treatment failure rate is reached, a switch to another more effective first-line drug is made [7].

Genetic markers for most forms of antimalarial resistance have now been described and include specific mutations in the propeller domain of the *Kelch13* gene associated with artemisinin resistance [9], and in the *plasmepsin 2-3* gene associated with piperaquine resistance [10]. Surveillance for resistance markers can be carried out by polymerase chain reaction (PCR) on dried blood spots collected on filter paper from a fingerprick. Genetic resistance testing is currently only used for research and surveillance

(http://www.wwarn.org/), although rapid advances in diagnostics technology mean that it may soon be technically possible to undertake point-of-care diagnosis in a clinical setting. *In-vitro* resistance, where cultured parasites are exposed to different concentrations of antimalarials, is restricted to highly specialised research laboratories.

Resistance to artemisinins and partner drugs

Artemisinin-resistant Pf was first documented on the Thai-Cambodian border in 2007-2008 [6], and is now found throughout most of the Great Mekong Sub-region (GMS) including in Vietnam, Myanmar and Laos [7, 11]. To date, although there are reports of ACT treatment failures elsewhere, artemisinin resistance has not yet been confirmed in Africa. However, with modern travel patterns, there are concerns that the spread of artemisinin-resistance is likely to be much faster than that of chloroquine-resistance, which also first emerged on the Thai-Cambodia border in the 1950s, reaching the East coast of Africa in the 1980s. If the spread of artemisinin resistance outpaces the speed at which a new class of antimalarials becomes available, the gains of the last 15 years will be lost and with it the hopes of eliminating malaria.

The situation in the GMS has become critical. Not only has resistance to the artemisinins spread geographically, but a specific resistant *Kelch13* haplotype (ie C580Y) is now becoming fixed in the parasite population and the emergence of resistance to the key partners drugs has also been confirmed [7]. In Cambodia treatment failure rates of around 40% to the first-line combination of dihydroartemisinin-piperaquine [12] forced a switch back to the previous first-line combination of artesunate and mefloquine which had only been switched from 4 years previously due to high levels of resistance. Fortunately there is some laboratory evidence to suggest that parasites which are resistant to mefloquine remain relatively sensitive to piperaquine and vice versa. The same phenomenon has also been observed between lumefantrine and amodiaquine, the

two main partner drugs used in Africa, neither of which however are effective in the GMS. Although this affords a little more buying time before a new class of antimalarials becomes available, the current pipeline will not bring a novel product to the market within the next 5 years (https://www.mmv.org/access/products-projects), and alternative approaches to deploy the current tools are being explored. This includes longer courses, the use of triple combinations containing artemisinin and two non-artemisinin partner drugs either at the same time or sequentially.

Treatment approaches in context of elimination

Elimination of malaria has now become a global health strategy in a number of regions including in the GMS, partly in response to the threat of artemisinin-resistance. Where there is a public health goal of transmission reduction in addition to individual patient cure, additional therapeutic approaches may apply. Treatment of Pf with single low dose (0.25mg/kg) primaquine is advocated in addition to an ACT, in order to clear gametocytes, the sexual form which does not cause symptoms but is responsible for transmission. The potential to exploit the mosquito killing properties of the anti-helminthic drug ivermectin is also being explored. At a population level, approaches to eliminate the malaria reservoir in asymptomatic carriers are being explored. These include mass drug administration and active screening and treatment using highly sensitive diagnostics aimed at detecting low-density infections.

Conclusion

The majority of malaria cases presenting to healthcare facilities in Europe are in people returning from visiting friends and relatives in Africa, where thankfully parasites remain sensitive to the artemisinins and the main partner drugs used in ACTs - including artemether-lumefantrine, dihydroartemisinin-piperaquine, artesunate-pyronaridine, the three fixed dosed ACTs that currently have EMA approval. These ACTs are safe and

effective against all types of malaria, not just *P. falciparum* malaria, making it possible to simplify treatment guidelines so that the first line treatment for uncomplicated malaria due to any species is an ACT, followed by primaquine for *P. vivax* or *P. ovale,* if not GP6D deficient. Quinine is still an effective drug and is a useful second line for severe malaria. Atovaqoune-proquanil (Malarone), which is primarily used for prophylaxis in travellers, also remains effective against Pf but its price and vulnerability to the development of resistance limits its use as a first-line agent in malaria-endemic countries.

This review has focused on the antimalarial resistance and treatment approaches, but prevention is better than cure. As health professionals we have a responsibility to ensure that patients receive risk-based pre-travel advice and where appropriate, effective antimalarial prophylaxis.

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