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REVIEWING ANTI-MALARIAL USAGE AND RESISTANCE PATTERNS AND ITS EFFECTS ON WORLD HEALTH ORGANISATION PROGRAMS

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Abstract

The two most significant strains of human malaria parasites responsible for morbidity and mortality are *Plasmodium falciparum* and *P. vivax*. One issue, which further compounds treatment of these pathogens, is one of drug resistance. Drug resistance often emerges from key mutations selected for by inadequate treatment regimes and has shown to be able to spread globally, further compounding the development of newer and more effective drug treatment programs, such as those from the World Health Organisation (WHO). Here we review the historical usage of anti-malarial drugs, the development of resistance in Africa and Asia, mechanisms of drug action and resistance, and the effects of resistance on WHO policy.

Keywords: malaria, drug-resistance, *Plasmodium*, World Health Organisation, drug policy.

Introduction

Drug resistant *Plasmodium falciparum* and *P. vivax* can be considered emerging infectious diseases because of the development of resistance mechanisms that decreases the amenability of the microorganisms to treatment, in addition to an increasing geographic range where these parasites are found [1].This complicates the treatment of malaria as well as threatening current World Health Organization (WHO) programs to eliminate malaria globally. Whilst five *Plasmodium* species cause malaria (*P. falciparum, P. vivax*, *P. ovale, P. malariae* and *P. knowlesi*) [2], only *P. falciparum* and *P. vivax* have clear evidence of robust resistance to current anti-malarials, as well as being the main contributors of disease morbidity and mortality [3, 4].

P. ovale and *P. malariae* have relatively unclear evidence of resistance, though there is some reported in the literature [5–7]. *P. knowlesi* is still zoonotic and less likely to be subject to drug selection pressures, in addition to continued susceptibility to chloroquine and mefloquine [8]. Further scientific work needs to be done on the non-*falciparum* species as far as resistance is concerned; however due to the aforementioned issue of morbidity and mortality, this review will focus on *P. falciparum* and *P. vivax*. Anti-malarial drug resistance is often defined in the literature by a combination of the presence of genetic markers, increased treatment times and increased *invitro* ring-stage survival assay half-lives(RSA) [1,9].

Molecular markers of resistance include *pfcrt* (*P. falciparum* chloroquine resistance transporter), *pfmrp* (multidrug resistance-associated protein), *pfmdr1* and *pfmdr2* (*P. falciparum* multidrug resistance transporter 1 and 2) and *pfnhe1(P.falciparum* sodium /proton exchanger 1) all of which encode for

transporter proteins [2]. Another molecular resistance motif includes antimalarial drug target modifications such as single nucleotide polymorphisms (snp) for the Kelch13 protein which confers artemisinin resistance(10)and mutations within the cytochrome *bc1* complex genes (e.g.*cyt b*) conferring atovaquone resistance [2]. Folate pathway anti-malarials (such as sulfadoxine-pyrimethamine) have also developed resistance genes via point mutations in both *pfdhfr* and *pfdhps* the genes encoding for dihydrofolate reductase-thymidylate synthase and dihydropteroate synthase respectively [2]. These gene complexes interact in a complex manner with the ecology of the parasite and human populations to produce geographical resistance patterns, which increase mortality and morbidity and cause problems for elimination programs for organizations like the WHO. This review will delve into the historical use of anti-malarials, subsequent development of resistance in Africa and Asia, mechanisms of drug action and resistance, and the effects of resistance on WHO policy.

Historical usage of anti-malarials for treatment and prophylaxis

Initially the fight against malaria was aimed at controlling the Anopheline and other mosquito vectors of the disease as famously discovered by Ross and Grassi in 1898. This includes disease transmission reduction strategies such as window and screen door installation, bed net distribution, reduction of mosquito breeding sites and widespread application of insecticides such as the largely used dichloro-phenyl-trichloroethane (DDT) [2]. This led to the elimination of malaria from over 10 countries from 1900 to 1946 (here it should be noted that Madagascar had a chloroquine chemoprophylaxis programme from 1945 to the 1960's [11]), and in 1955 the WHO launched the Global Malaria Eradication Programme where chloroquine was administered as a monotherapy to complement initial vector measures, which when ended in 1969 saw 27 more countries declared free of malaria [2]. However this long term programme was only partially successful as most underdeveloped countries did not achieve malaria elimination and Sub-Saharan Africa was not included in the original malaria eradication programme which accounts for the current distribution of malaria in subtropical and tropical regions [2]. Widespread resistance to the pesticides of the time, wars, massive population movement, difficulties with obtaining sustained funding and the emergence and spread of chloroquine resistance led to a halt in this programme and at the time the only viable antimalarial treatment was sulfadoxine-pyrimethamine; however this also encountered resistance a year after it was administered [2]. Mefloquine, amodiaquine and quinine also followed the same path being associated with monotherapy [2]. In 1998 malaria elimination was reattempted with relative success with reductions of 20% from 985 000 in 2000 to 781 000 in 2009 with respect to malaria related mortality [2] (this estimate is one of many, see Murray C.J., *et al.* 2012 for an exploration of the issues associated with mortality estimates as these will vary depending on the information base and criteria used to generate them [12]).Madagascar's 60 years of experience (1945–2005) with chloroquine demonstrates some of the organisational challenges of maintaining an effective elimination program over a long period of time with funding problems, supply chain issues, degradation of health care systems and various governmental issues complicating and reducing the efficacy of the elimination programme [11]. These issues plus evolutionary reactions to the elimination programs from the *Plasmodium* species in question lead to a resurgence in malaria in the 1980's as well as an increase in mortality and morbidity from malaria in both Madagascar and the rest of the malaria-affected parts of the world [2, 11]. Current geographic prevalence patterns and differences in resistance reflect this history and show that scientific advances often have a time limit attached with respect to prophylaxis and treatment of malaria.

Anti-Malarial Resistance in Africa and Asia

It has been shown in both key African nations including Burkina Faso, Gambia, Ghana and Mali, and within Asia (Thailand, Vietnam and Cambodia; particularly Western Cambodia), that *P. falciparum* has utilized an unusual but nonetheless effective parasite population structure whereby discrete but different parasite subpopulations, each with a great degree of genetic variation, exist in a small geographic area by way of irregularly high levels of haplotype homozygosity as well astransporter protein codon variants [13]. These transporter proteins play a vital role in anti-malarial resistance and include the ABC transporter of the heavy metal transporter family, the multidrug resistance protein (MDR1), a formate-nitrite transporter, and cation-transporting ATPase [13]. Further resistance has been augmented by sexual recombination, which occasionally produces an optimal combination of alleles that confers both efficient drug resistance [13]. In particular, three of these subpopulations in Western Cambodia (Pailin, Tasanh and Pursat) discussed by Miotto *et al.* have developed resistance to artemisinin, which itself was utilized as a replacement to its predecessor chloroquine in malaria first line therapy [14], outlining a need for an intermediate to long term frontline drug solution before a long term solution in the form of a vaccine will materialize. It is noted that these regions are isolated, making them optimal locations for

parasite inbreeding, thereby enhancing the vector mechanism where parasite subpopulations are preferentially transmitted by different species of *Anopheles*, reinforcing an optimal allele combination for drug resistance [14]. It should also be noted that this general pathway to resistance, which includes transporter proteins and parasite subpopulation inbreeding is how resistance to chloroquine originated and now is a conspicuous fact driving resistance to artemisinin [15]. Another major factor in artemisinin resistance is continued poor application of artemisinin monotherapies since the mid 1970's in the form of sub-therapeutic doses and artemisinins which are not of therapeutic quality (i.e. fake drugs) [16], allowing resistance to propagate and evolve where there is mild selection pressure over time in a non-lethal environment.

Artemisinin resistance was first reported in Western Cambodia [17] and it is now prevalent across mainland South-East Asiaincluding Myanmar, Cambodia, Laos, Thailand [1, 18–21], with another recent study looking at 10 countries (see figure 1 of ref [1]). Of the African countries examined Nigeria, Kenya and the Democratic Republic of Congo showed no resistance to artemisinin, with Nigeria and the Democratic Republic of Congo having some limited clearance rates above five hours but no detectable Kelch13 polymorphisms at or beyond amino acid position 441(a substitution of a proline to a leucine [1]).Here it should be noted the major drivers of anti-malarial resistance are listed in Table1 below.

Table 1

Major factors contributing to malaria's resistance to drugs.

Drivers of anti-malaria resistance	References
1) Widespread availability of artemisinin monotherapies	(22, 23)
2) Poor quality artemisinin-based combination therapies (ACT) including counterfeit antimalarial drugs	(24, 25)
3) Monotherapies containing sub therapeutic amounts of ingredients and unregulated use of anti-malarial agents	(24, 25)
4) Poor quality anti-malarial drugs (besides artemisinin) containing sub-clinical doses of active drugs	(26, 27)
5) Unusual genetic structure of *Plasmodium* populations in this region	(1)

The interaction between the unique population genetics of *Plasmodium* species in Cambodia and the preceding factors seem to make it an epicentre for resistance as it was here that most of the successive waves of resistance (including other antimalarials like chloroquine, sulfadoxine and pyrimethamine) appear to have originated [13]. Due to longer exposure duration, non-artemisinin based anti-malarial resistance patterns (see figure 2 inref [1]) like chloroquine, amodiaquine and sulfadoxine-pyrimethamine have a wider spread distribution [2]. For instance, although many of these resistance patterns had origins in South East Asia (in the 1960's for chloroquine), chloroquine resistant *P. falciparum* then spread to Africa, which in combination with no affordable effective alternative lead to a two- to three-fold increase in malaria-related deaths in the 1980's [28]. This well-known pattern of spreading drug resistance along with new evidence of limited polymorphisms in *P. vivax* dihydrofolate reductase genes (suggesting changes in other genes as responsible for drug resistance) demonstrates an expanding geographical range for these resistance genes [29].

Mechanisms of drug action and resistance

Plasmodium parasites are peculiar organisms, possessing different life cycle stages, each highly adapted to the various cellular environments in which they are found; the cycling of these different morphological states has made a complete radical cure elusive [30].

The first effective treatment came from drugs synthesised from the bark of the *Cinchona* tree namely chloroquine [31]. Chloroquine's method of function is to cause haem toxicity by inhibiting *Plasmodium* from converting dimers of Fe(III) PPIX (haematin) into crystals of haemozoin which eventually causes membrane damage and lysis [32]. However resistant strains arose out of chloroquine's ubiquitous use, giving rise to a mutation in the *pfcrt* gene, which codes for a chloroquine resistance transporter protein (PfCRT) on the membrane of the digestive vacuole (DV) allowing chloroquine concentrations to decrease [32]. This prompted development of new derivatives of chloroquine, but with varying efficacy and each complicated by additional resistance, toxicity and patient compliance [32]. Derivatives of quinine compounds such as mefloquine were effective but gave rise to untenable side effects which made it unsuitable for some cases, and they too became the subject of resistance from *Plasmodium* parasites again via a mutation in the *pfmdr1* gene which codes for a transporter with analogous functions to PfCRT [32].

With the *Cinchona* bark derivatives being found to be limited due to the factors stated above another possible solution was from artemisinins, the active anti-malarial compound also known as «Qinghaosu», or the «Sweet Wormwood» [32]. Artemisinins act by releasing carbon-centred radicals that target the structures of the cell, specifically cellular lipids, DV membranes, inactivating proteins, and like the *Cinchona* derivatives – interfering with the production of hemozoins [32]. Currently artemisinins are the frontline therapy for *P. falciparum*, but again resistance in the species has arisen through strains that have PfATP6 mutations (a single amino acid mutation, Leu263) [33], as well as those strains lacking mutations in the genes *atp6* and *mdr1*, suggesting a non-mutually exclusive alternative mechanism [31].

Resistance arising from target modification and transporter proteins

Malaria resistance can also be classified via two main mechanisms; firstly, those that are target modification related such as the Kelch13 propeller single nucleotide polymorphisms with artemisinin, and folate pathway enzyme modification (e.g. sulphadoxamine-pyrimethamine resistance) [34]. Secondly, those that are transporter protein related such as chloroquine resistance and multidrug resistance [35, 36]. The cellular mechanisms of Kelch13 artemisinin resistance are relatively unknown, as the gene has only recently been discovered [9]. In 2014, Ariey *et al.* stated that according to homology studies, the Kelch13 mutations destabilise the protein domain scaffold and alter its function [9]. Based on the premise that the toxicity of artemisinin is reactive oxygen species (ROS) dependent (although other mechanisms have been postulated [35]), its function in regulating cytoprotective and degradative responses to external stress is of interest [9]. A recent study by Mbengue *et al.* [37] demonstrated a biochemical mechanism for the resistance to artemisinin by *Plasmodium* parasites. Briefly, in non-resistant parasites, artemisinininhibitsphosphatidylinositol-3-kinase (PI3K), leading to a decreased production of phosphatidylinositol-3-phosphate (PI3P). The PI3P molecule has an important role in parasite protein export with infected erythrocytes, as shown in prior work by the same group [38].The researchers propose that, in wild-type parasites, Kelch13 binds to PI3K, leading to ubiquination and protein degradation. However, in artemisinin resistant strains, the Kelch13 C580Y mutation confers less binding of the Kelch13 protein to PI3K, leading to increase in PI3K levels and therefore, an increase in PI3P in the parasite [37]. Furthermore, introducing wild-type Kelch13 into resistant parasites resulted in a reduction of PI3P and resistance (measured as a decrease in RSA). Likewise, the introduction of Kelch13 C580Y into sensitive parasites resulted in an increase in PI3P and resistance (measured as an increase in ring-stage survival assay). Overall this study demonstrated a connection between Kelch13 C580Y mutation, PI3K and its product PI3P, in conferring resistance to artemisinin.

More is known about non-Kelch13 forms of resistance as they have been studied more extensively. Transporter proteins are largely associated with DV of *Plasmodium* species, with chloroquine and other quinine-based synthetics being shown to bind to haem and prevent its detoxification process [2]. For example, transporter proteins can reduce the concentration of quinine derivatives such as chloroquine within the DV and thus interfere with their action. Other proteins such as the glycoprotein coded by the *fpmdr1* gene (a 162 kDa protein with a structure similar to the ATP binding cassette [ABC] family of transporters and glycoproteins) have also been postulated to interact with other resistance genes and proteins [36]. The folate pathway of anti-malarial resistance arises via the target modification of the dihydropteroate synthase and dihydrofolate reductase enzymes (due to point mutations in both genes that encode these enzymes) which reduce the chemical binding affinity of the anti-malarials for those enzymes [36]. This mechanism confers resistance to sulfadoxine, dapsone, and pyrimethamine and proguanil [36].

In a recent study utilising the *P. berghei*mouse model of malaria by Lin *et al.* [39], researchers created parasites with mutations in enzymes that are involved in haemoglobin digestion, specifically plasmepsin 4 (PM4) and berghepain 2 (BP2), and that with these mutations, they were limited to reticulocytes and were of smaller size compared to wild-type parasites, across different stages of the lifecycle. The most surprising finding by the researchers was that, although impaired in haemoglobin digestion, these mutant parasites were resistant to chloroquine, even at high doses. This observation indicates a new mechanism of resistance to compounds that specifically target haemoglobin digestion and could have potential relevance to *P. vivax* infections, given that *berghei*, like *vivax*, is also reticulocyte-restricted, and that resistance to chloroquine by *vivax* differs to that of *falciparum* (that is, *falciparum* resistance to chloroquine is through mutations in the *pfcrt* and *pfmdr1* genes) [39]. How well this observation occurs in chloroquine-resistant *P. vivax* infections remains to be elucidated. Interestingly, the chloroquine-resistant parasites remained sensitive to artesunate, further highlighting the importance of treating cases of chloroquine-resistance with artemisinin-combination therapies.

Further complicating drug-resistance is the findings from a study by Regev–Rudzki *et al.* [40], demonstrating that *P. falciparum* infected erythrocytes can communicate between each other, using

exosome-like vesicles. In particular, they were able to demonstrate that genes conferring resistance to drugs were able to transmit between infected erythrocytes. The spread of resistance between infected erythrocytes will be another factor to address in the problem of spreading drug resistance.

Addressing resistance pharmacologically

Given that the use of chloroquine, artemisinin and their common derivatives has given rise to resistance, new drugs are being evaluated such as ferroquine, pyronaridine, piperaquine [41] and quinolone-3-diarylethers [42]. Ferroquine, which has undergone successful Phase I trials [43], is proposed to counter chloroquine resistance [44, 45] by utilizing a modified functional group at the 4-amino position [30]. Its mechanism is characterised by disrupting membrane proteins and the arresting of haemozoin formation [30]. Pyronaridine also contains a variation on the 4-amino acid side chain and has entered Phase III clinical trials [46]; however this variation on the side chain determines a different mechanism of action whereby deleterious modifications on the DV of late trophozoites and schizoints as well as the acting on haematin formation have made this a viable option [47] (in this way operating similarly to its predecessor chloroquine) as well as in combination therapy with artesunate [48]. Piperaquine was also shown to be effective until resistance developed in China [31] and now is used with some effectiveness as a combination therapy with dihydroartemisinin [49]. However although the mechanism of piperaquine has not been fully elucidated, its structure is characterised by a 4-amino group modification and may share a similar mechanism of action as chloroquine and pyronaridine.

A new class of drug which has been put forward in 2013 were the quinolone-3-diarylethers, specifically ELQ-300 [42]. It has been shown that ELQ-300 targets the Q_i site of the both the *P. falciparum* and *P. vivax* mitochondrial enzyme complex known as cytochrome bc_1 [42]. ELQ-300 targets the liver and blood stages, as well has being effective against the exoerythrocitic stages, schizonts, and inhibiting the formation of ookinetes and ooctyes [42]. Its mechanism of action greatly differs from those of the chloroquine derivatives outlined above (where the pathway involving the DV is targeted); here parasite oxygen respiration is rapidly inhibited [42]. Further, in order to test for the possibility of resistance to this drug in the future an attempt to generate resistant mutants was made and failed, suggesting that the development of resistance may not be as frequent compared to chloroquine, its derivatives and artemisinin [42]. This particular study with ELQ-300 was done both *in vitro* and *in vivo*, showing good bioavailability in therapeutically relevant doses [42] and if this drug did proceed to Phase I human clinical trials it would provide a shift away from the traditional drugs outlined previously.

Effects of malaria resistance on World Health Organisationmalarial elimination programmes

Section 6 of the WHO World Malaria Report 2014 [50] and other WHO reports [22, 51, 52], discuss the current concern of *P. falciparum* resistance to drugs, in particular to artemisinin, which has emerged in countries that constitute the Greater Mekong Subregion, such as Myanmar and Thailand, as well as parts of South America.

In the majority of countries, the WHO recommends a three-day course ofan ACT for uncomplicated malaria, which involves a fast acting artemisinin compound, in combination with a second, longer lasting anti-malarial substance such as mefloquine. Since 2013, ACTs are utilised as a first-line treatment option in 79 of 87 countries [50]. However the long-term utility of ACTs is threatened by the emergence of artemisinin resistance in places like the Greater Mekong Subregion where it may spread. ACT utility is also threatened by the use of artemisinin monotherapies, which involve a 7 day treatment regime (compared to 3 days with an ACT), but causes many patients to drop out of the treatment program early, thereby encouraging resistance to continue [22].

In order to prolong the long term effectiveness of ACTs as useful treatment, the WHO is recommending that a majority of countries to cease the use and marketing of artemisinin monotherapies, in both public and private sectors, and to encourage the proper use of the ACTs [22, 50, 52]. This in itself presents its own challenges, because the plan will involve targeting all facets of the production cycle of artemisinin monotherapies, the complex dynamics of international trade and in the enforcement of regulations by states. The WHO nevertheless gives a generic framework for accomplishing this task of phasing out artemisinin monotherapies [22]. To further assist in maintaining anti-malarial effectiveness, the WHO also recommends that countries perform Therapeutic Efficacy Studies (TES) every two years at sentinel sites, which would serve to guide treatment regimes and to monitor for suspected cases of resistance to anti-malarials, particularly artemisinin.

While maintaining the effectiveness of ACTs is vital via phasing out artemisinin monotherapies, it is also important to stop the movement of these resistance mechanisms. The WHO developed in 2013 the "Emergency response to artemisinin resistance (ERAR) in the Greater Mekong subregion: A regional framework for action 2013–2015" [53], a follow up of the 2011 "Global plan for artemisinin resistance

containment (GPARC)" report [54]. As a framework, this emergency plan provides recommendations to contain the spread of resistance to other locations in the Greater Mekong Subregion, which the WHO considers a feasible goal [50].

The WHO emphasises that, without combating these practices that contribute to resistance spreading, the first line of defence against uncomplicated malaria, the ACT, would lose its therapeutic ability. With no safe and effective alternative to replace artemisinin in the next few years, the public health risk is even greater. A recent article by Lubell and collegues [55] models the economic and human cost of artemisinin resistance, with an increase in mortality of approximately 100,000 people per year, with productively losses of US$385 million and increases in medical costs of US$32 million per year. These "ballpark" estimates are a cause for concern. However, the good news is that from past experience, the removal of a drug pressure in an area of resistance, these resistance genes confer reduced survival and fitness to parasites relative to non-resistance parasites, which can serve to increase the life span of the corresponding drug [22, 56].

Conclusions

Considering that artemisinin and other antimalarial drugs such as quinine and chloroquine resistance is already well established in South east Asia, with non-artemisinin based anti-malarials spread around the world [22, 57] and that artemisinin is a last line of chemotherapy for *P. falciparum*, a vigorous response and containment policy is required to avoid spreading of these resistance genes amongst *Plasmodium* species [22, 50]. Surveillance programs for resistance need to not only take into consideration the major species like *P. vivax* and *P. falciparum* but also need to monitor the other 3 *Plasmodium* species as well to avoid a disease succession scenario where one form of the disease becomes predominate as another one wanes (i.e. *P. knowlesi)*. The scope of current malaria control research is very diverse and with the addition of molecular methodologies and the sequencing of the *P. falciparum* and *P. vivax* genomes providing new targets for chemical and treatment control as well as new tools in terms of molecular markers (surveillance) have become available as a result of increasing understanding of the disease agents and resistance molecular mechanisms [58–60].

Part of the problem with artemisinin resistance is that it is a recent phenomenon and current knowledge is far from being comprehensive in both the artemisinin mechanisms of action and the resistance mechanisms. Diverse approaches involving changing prescription behaviours, as proposed by the WHO through policy and legislative means [22], encouraging more malaria control and surveillance research and altering the balance of activity of anti-malarial programs based on the on-going surveillance results. For example a staggered complementary approach could be used if artemisinin resistance surveillance shows an increase in prevalence then a switch to vector control or transmission could be used in addition to evaluating the need for different prescribing approaches of other anti-malarials. This approach could use the assumed fitness cost of the resistance mechanisms to advantage assuming that compensatory mutations have not occurred. The emergence and spread of malaria resistance is a clear and present danger to the progress made in recent years by the WHO on their ability to control the spread and damage caused by malaria.

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ОБЗОР ПРИМЕНЕНИЯ ПРОТИВОМАЛЯРИЙНЫХ ПРЕПАРАТОВ, ПРИМЕРЫ ЛЕКАРСТВЕННОЙ УСТОЙЧИВОСТИ И ЕЕ ВЛИЯНИЕ НА ЭФФЕКТИВНОСТЬ ПРОГРАММ ВСЕМИРНОЙ ОРГАНИЗАЦИИ ЗДРАВООХРАНЕНИЯ (ВОЗ)

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Реферат

Два наиболее распространенных штамма паразитов, вызывающие малярию у человека, – это *Plasmodium falciparum* и *P. vivax.*

Проблема, касающаяся лечения этих болезней, это проблема устойчивости к лекарственным препаратам. Устойчивость к лекарственным препаратам часто возникает вследствие ключевых мутаций при неправильном выборе метода лечения, и уже продемонстрировала свою способность к глобальному распространению, затрудняя дальнейшую разработку современных и более эффективных лечебных программ, таких как программы Всемирной Организации Здравоохранения (ВОЗ). В этой статье мы рассматриваем историю применения противомалярийных средств, развитие устойчивости к ним в Африке и Азии, механизм действия лекарственных препаратов и лекарственную устойчивость, а также влияние устойчивости на политику ВОЗ.

Ключевые слова: малярия, лекарственная устойчивость.