Original article:

Drug resistant plasmodium falciparum parasites: a review of the resistance and failure of malaria eradication

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Abstract:

Malaria infection remains the leading vector borne disease in the world today. Given the increasing report of resistance or poor responses to artemisinin based combination therapies (ACTS), the sub-Saharan African region affected by the disease might receive a repeat of what happened during the emergence of chloroquine and sulfadoxine pyremethamine resistance. If such case arises, the malaria control efforts in the region may be compromised and the little success gained of intervention efforts may be eroded. Although the world has currently embraced the use of recommended artemisinin combination based therapies (ACTS) for the treatment of uncomplicated p. malaria. Current assessment of drug susceptibility and level of circulating resistant Plasmodium parasites is not full elucidated. Assessment of *P. falciparum* is thus necessary to sustain quality control programmes, appropriate use of therapy, and health policy advice in respect of malaria management in countries where malaria is endemic. This current paper brings out the challenge of antimalarial resistance in malaria eradication agenda.

Key words: Malaria, antimalarial, Plasmodium.

1. Introduction.

Malaria is a disease caused by a protozoan parasite belonging to the phylum of apicomplexia. There are five main species causing malaria; these are *Plasmodium ovale, P.* vivax, *P. malariae*, *P. falciparum and P. knowlesi*. Out of these *P. falciparum* has been documented to cause more deaths annually in endemic areas of sub-Saharan Africa (1) . Recently mortality from malaria has reduced due to the use of artemisinin based

combination therapies (ACTS) and other control measures.

To date, drug resistance has been documented in three of the five species namely; *P. falciparum, P.malariae* and *P. vivax*. However, P. falciparum resistance is emerging to artemisinin based combination therapies (ACTS). The artemisinin-based combination therapies were introduced in the mid-1990s when there was a challenge of untreatable malaria in Southeast Asia, where resistance to all available antimalarial drugs had developed. In 2005, the World Health Organization recommended that artemisinin-based combination therapies be used as first-line treatments for *P.falciparum* malaria in all countries where malaria is endemic (2).

According to the WHO World Malaria Report 2014, 198 million cases of malaria were recorded globally in 2013 and the disease led to 584,000 deaths. The burden is heaviest in sub-Saharan Africa, where an estimated 90% of all malaria deaths occur. Children aged less than five years account for 78% of all malaria deaths. It has been reported by that over 90 % of the African population are at risk of the infection for example it was reported from Uganda, that in 2013 alone, 1,502,362 of 3,718,588 Ugandans reporting at health facilities and examined for malaria parasite infection were confirmed positive (3). However several cases occur outside health facilities and treated with unknown outcomes.

2 Antimalaria drugs and resistance *of Plasmodium. falciparum.*

The commonly used drugs for the treatment of malaria include**:** Antifolates, Quinoline derivatives, Artemisinin-based combination therapies (ACTs).

2.1Antifolates.

Antifolates are the antimalarial drugs which were developed after the quinolines were found to be resistant by the malaria parasites. These drugs are currently used in parts of the world; however resistance has been reported in some parts of the world especially in the African continent (4). Antifolates are subdivided into two classes viz; dihydropteroate synthase (dhps) inhibitors and dihydrofolate reductase (dhfr) inhibitors, depending on the enzymes they inhibit in folate metabolism pathway. The major advantage of the Antifolates is that they attack all growing stages of malaria parasites. The most commonly used antifolate drugs for treatment of malaria include: pyrimethamine/sulfadoxine, sulfalene/pyrimethamine, dapsone/pyrimethamine, and chlorproguanil/dapsone (5) .

2.2 Quinoline derivatives.

This have been regarded as the first generation antimalarial drugs which are composed of chloroquine(CQ), quinine(QN), amodiaquine(AQ) and mefloquine (MEF).They were the first ones to be introduced for the public use, however the malarial parasites are rapidly becoming resistant to these antimalarial agents.Chloroquine, a 4-aminoquinoline compound was introduced in 1944–1945 and became the mainstay of therapy and prevention, however there has been reports of CQ resistance . Amodiaquine is chemically related to CQ, but is more effective than CQ in clearing malaria parasites in patients with uncomplicated malaria and those affected with CQ-resistant strains (7) . Although drug resistance and potential hepatic toxicity limit it's use, it still remains treatment of choice. Quinine a quinoline-methanol which was isolated from the bark of *Peruvian cinchona* trees in China in the 17th century remains an essential antimalarial drug for severe *P. falciparum* malaria and intravenous infusion (8). Lumefantrine is closely related to

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halofantrine, but it is potent against malaria although is limited due to it's serious cardiac toxicity. It's biological activity resembles the class-2 aryl amino-alcohols (in which the quinoline portion of the 4-quinoline methanol is replaced by a different aromatic ring system). It is a highly lipophilic compound. Lumefantrine in combination with artemether shows synergistic activity with high potency. This combination is now recommended as the first line treatment for uncomplicated malaria in many African countries.

Piperaquine (PIP) is a bisquinoline with two quinoline nuclei bound by a covalent aliphatic chain. It was identified as a promising candidate during drug screening programs in the 1960s. It was then used as monotherapy for *P. falciparum* malaria in China until the development of drug resistance (9). Piperaquine is a highly lipid-soluble drug, well distributed in the body and has a long elimination half-life and better clearance in children than in adults. The tolerability, efficacy and low cost of piperaquine make it a promising drug for use. Piperaquine was re-evaluated in the 1990's and shown to be active in vitro against chloroquine-resistant *P. falciparum* isolates (10).

2.3 Artemisinin-based combination therapies (ACTs)

WHO recommends artemisinin-based combination therapies (ACTs) for the treatment of uncomplicated malaria caused by *Plasmodium* parasites in most countries where resistance has evolved towards other drugs. The current ACT regimens include: artemether/lumefantrine, artesunate/amodiaquine, artesunate/mefloquine, sulfadoxine/pyrimethamine-artesunate and dihydro artemisinin/piperaquine (11) . ACTs comprises of artemisinin derivatives with short plasma elimination half-lives, combined with a longer-acting partner drug (Ashley and White.,2005). ACTs are being evaluated in many clinical trials in Africa. Previous studies in Uganda reported the occurrence of recrudescence after ACT treatments ranging from 1- 12% after 28 day follow up (12) . A previous study conducted in Tororo, Uganda (13), comparing Coartem with AQ/AS reported recrudesce rates of 8.4% and 4.2% , and new infections within 28 days of treatment in 66% and 55% of subjects. In another study in Tororo (14) concluded that there was selection of three polymorphisms related with diminished response to lumefantrine in the pfmdr-1 gene after administration of Artemisinin Lumefantrine. Similar findings were reported in Tanzania (15) found that Coartem selected pfmdr-1 polymorphisms genes which were associated with diminished sensitivity to lumefantrine. The following five ACTs are recommended for malaria treatment: artemether + lumefantrine, artesunate+amodiaquine, artesuna-te+mefloquine,

artesunate+sulfadoxinepyrimethamine, artemisinin + piperaquine. To reduce the risk of development of resistance to artemisinin-based combination therapies (ACTs),WHO discourages the use of oral artemisinin-based monotherapy drugs but instead use ACTs.

3. Antimalarial resistance genes.

Antimalarial resistance genes are the molecular markers which have been found to be conferring the properties of resistance. They can be used to design strategies of early diagnosis of resistance parasites to avoid earlier drug failure in proper medical interventions . The following are some of the resistance genes which can be used as the resistance molecular markers respectively: *P. falciparum* chloroquine resistant gene (pfcrt), *P. falciparum*

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multidrug resistance gene (pfmdr-1, *P. falciparum* transporter sarco/endoplasmic reticulum (Pfatpase6) resistance gene, *Plasmodium falciparum* dihydrofolate reductase (dhfr) and *P. falciparum* dihydropteroate synthase (dhps) genes.

3.1 *Plasmodium falciparum* chloroquine resistant gene (Pfcrt).

The Pfcrt resistant gene is usually located on chromosome 7. The components of this gene are the 13 axons which encodes the membrane protein, present in the parasite's digestive vacuole during the erythrocytic phase of the human cycle. This resistant gene (pfcrt) belongs to the metabolite transporter super family (16). The resistance has developed due to point mutations occurring at position 20 in the gene. This phenomenon is as a result of substitution of threonine (T) for lysine (K) at position 76 (K76T). However some previous studies (17) reported that some parasites with the 76T allele of pfcrt were not resistant to chloroquine. This finding however was not conclusive since it may have been due to the mixed infections in vivo or new compensatory gene mutation. A study in Uganda (18) found out that there was 100% prevalence of the 76T mutation with other specific single nucleotide polymorphisms (SNPs) at positions 72 –76 form haplotypes that have been implicated in the chloroquine resistant strains.

Haplotypes of 9 CVIET isolated in Asia and Africa and SVMNT isolated from South America have been associated with CQ resistance (19). Pfcrt polymorphisms may also play a role in parasite response to other antimalarial drugs. It was observed in (20) studies that, substitutions at position 76 (K76I or K76N) does not only promote resistance to CQ and AQ, but sometimes makes the malaria parasite to be sensitive to quinine, mefloquine, halofantrine and artemisinin.

3.2 Plasmodium falciparum multidrug resistance gene (pfmdr-1).

pfmdr-1 gene is usually located on chromosome 5, a transmembrane protein which encodes a protein ,P glycoprotein homologue (Pgh-1) which belongs to the ABC transporter family (21). The P glycoprotein is found in the malaria parasite digestive vacuole, the target site for action of chloroquine and quinoline-based antimalarial drugs such as amodoquine and Quinine (22). Previous studies have found out that five different mutations in pfmdr-1 have been associated with drug resistance to quinine, halofantrine and artemisinin derivatives at points of N86Y, F184Y, S1034C, N1042D, and D1246Y (23).

Plasmodium falciparum multi drug resistance increased copy number of Pfmdr-1gene amplification was found to be directly related to the parasite responses to quinine, lumefantrine mefloquine and artemisinins derivatives respectively however this was not related to Chloroquine and Amodiquine (23). The gene alterations in pfmdr-1 copy number in most of Africa countries compared to Asia. Despite of the fact that pfmdr-1 and pfcrt resistance genes are found on the chromosomes 7 and 5, some studies have indicated that there is a strong relationship between polymorphisms in the two genes (24).It has also been observed that there is a clear relationship between pfmdr-1 N86Y and pfcrt K76T in some malaria parasites (25). Pfcrt and pfmdr-1 genes single nucleotide polymorphism and mutational changes occurring in the copy number have been found to affect parasite sensitivity to mefloquine, artemisinin and quinine (26). Consequently a study by (27) concluded that some regions in pfmdr-1, pfcrt and pfnhe1 on chromosomes 5, 7 and 13 were promoting quinine resistance.

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3.3 *Plasmodium falciparum* dihydrofolate reductase (dhfr) and *Plasmodium falciparum* dihydropteroate synthase (dhps) genes.

These specific resistance genes are correlated to *P. falciparum* enzymes which are target site for antifolates such as sulfodoxine pyrimethamine (SP). In *P. falciparum* SP resistance is associated with point mutations occurring at dihydrofolate reductase (pf-dhfr) and *P. falciparum* dihydropteroate synthase (pf-dhps) mutation points (29). The pf-dhfr, point mutations occurs as a result of changes in Asn51 to Ile (N51I), Cys59 to Arg (C59R), Ser108 to Asn (S108N), and Ile164 to Leu (I164L) .These changes promote resistance to pyrimethamine (30). The resistance conferred by malaria parasites to Sulfadoxine is due to dhps mutations occurring at codons 436 (S436A/F), 437 (A437G), 540 (K540E), 581 (A581G), and 613 (A613S/T) (31).

3.4 *Plasmodium falciparum* transporter sarco/endoplasmic reticulum (Pfatpase6) resistance gene.

PFATPase6 resistance genes encodes for sarco/endoplasmic reticulum Ca2+-ATPase (SERCA) which is a calcium transporter. It has been observed previously that this calcium transporter may be targets of artemisinin derivatives (32). Moreover other studies have indicated that artemisinin may inhibit ATPase and alter intracellular calcium stores (33). Another previous study concluded that mutation I89T in PfATPase6 were present in parasites which were sensitive to artemsinins (34). However it has also been observed that gene polymorphism occurring at PFATPase6 S769N, promoted artemether resistance in malaria parasites (35).

4. Antimalarial susceptibility tests.

These are the methods which are used to monitor the response of malaria parasites to antimalarial drugs. This can be done by invitro, invivo and molecular methods respectively.

4.1 Invivo methods.

The invivo methods involves the treatment of individuals who are symptomatic and parasitaemic by using known doses of drug and then monitoring the clinical response and parasitological clearance over a period of time using standard methods. The follow up period varies from one endemic region to another; however the standard gold follow up time recommended is a minimum of 28 days **.**The advantage of invivo tests is that they usually reflect actual clinical or epidemiological situations in relation to the therapeutic response of currently circulating parasites. The invivo test gives the actual results of the efficacy of antimalarial treatment in relation to the clinical status. The major drawback of this method is that sometimes the results are affected by the major differences in enrolment criteria, sample size, exclusion criteria, and follow up duration (36). Additionally there is a problem in differentiating between the resistant strains and new infections. However the World Health Organization (WHO) has recommended that: Children (younger than 5 years) with clinical malaria should be the study subjects in all areas but where the transmission is low all ages can be enrolled (37).

4.**2 Invitro methods**

In vitro drug method involves the use of laboratory based methods to monitor trends of antimalarial drug susceptibility. They are based on measurement of the effect of drugs on the growth and development of malaria parasites. These methods are used to detect the early stages of resistance as an alternative tool for the surveillance of drug resistance. Culturing of *P.falciparum* started as early as 1970s with the work of Trager and Jensen (1976) who demonstrated that

P. falciparum infected erythrocytes can be cultured. Invitro test can show parasite responses to drugs without any interference of host factors, immunity, and patient compliance to the drug treatment. The current protocols used include: WHO microtest /Schizont maturation assays, lactate dehydrogenase (pLDH), and histidine-rich protein 2(HRP2), SYBER Green1 and other fluorescent dyes isotopic (tritiated hypoxanthine uptake) assay. However the recommended method that is used is the WHO micro test. In WHO micro test, blood sample is obtained from the malaria patient as per the standard protocol. Different concentrations of the antimalaria agents are made. The samples are incubated together with the antimalaria agents at 37ºC for 72 hours. Mature Schizonts of the parasites are observed by using microscopy (38).

4.3 Molecular methods

The molecular methods are used to detect the presence of the resistance genes which are used as molecular markers. They indicate the presence of mutations encoding biological resistance to antimalarial drugs. Molecular tests used include the restriction fragment length polymorphism (RFLP) and polymerase chain reaction (PCR) (39).

Conclusion

Malaria still remains a major challenge in the public health sector. More over this has been augmented by the development and spread of the drug resistant plasmodium parasites. Thus continuous surveillance and mapping of the resistance patterns is essential so as to promote containment. Malaria is a multifaceted disease that changes from one geographical location to another in relation to clinical outcomes and epidemiology. This inconsistency is as the outcome of profounding factors such as the circulation and competence of mosquito vectors, the malaria parasites species, their susceptibility patterns to antimalarial agents and environmental factors

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