

**Review Article****Open Access****Artemisinin drug resistance and monitoring: a narrative review**

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Abstract:

Artemisinin drug resistance is one of the major reasons for malaria treatment failures in the sub-Saharan African countries where artemisinin-based combination therapy (ACT) is the first-line treatment for uncomplicated malaria. The occurrence of single nucleotide polymorphisms (SNPs) is found to correlate with antimalarial drug resistance. With artemisinin, the SNPs occurs at the Kelch 13-propeller gene locus on chromosome 13. The artemisinin drug resistance surveillance strategy involves continuous monitoring of Kelch 13-propeller biomarker to detect emergence of mutations which could herald drug resistance in the region. In this narrative review paper, we examined existing literature to bridge the knowledge gap and accentuate the importance of routine surveillance for artemisinin resistance in sub-Saharan Africa. We conducted our search on PubMed database and Google Scholar to identify peer-reviewed articles, reports, and abstracts on artemisinin drug resistance using the following keywords; 'artemisinin drug resistance', 'antimalarial drug resistance', 'artemisinin-based combination therapy', 'Kelch 13-propeller', 'K13-propeller gene', and 'K13 molecular marker'. The review provided pertinent information on artemisinin derivatives, artemisinin-based combination therapy, molecular action of artemisinin, definition of artemisinin resistance, genetic basis of artemisinin drug resistance and discovery of Kelch 13, and the importance of artemisinin resistance surveillance. Molecular surveillance can provide healthcare policy makers a forecast of impending threats to malaria treatment. This is more so when drugs are in combination therapy, for instance, molecular surveillance can give a hint that one drug is failing despite the fact that in combination, it is still apparently clinically effective.

Keywords: malaria; artemisinin; resistance; molecular marker; Kelch 13-propeller gene

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Résistance aux médicaments à base d'artémisinine et surveillance: un examen narratif

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Résumé:

La résistance aux médicaments à base d'artémisinine est l'une des principales raisons des échecs du traitement du paludisme dans les pays d'Afrique subsaharienne où la polythérapie à base d'artémisinine (ACT) est le traitement de première intention du paludisme simple. L'apparition de polymorphismes mononucléotidiques (SNP) est corrélée à la résistance aux médicaments antipaludiques. Avec l'artémisinine, les SNP se produisent au locus du gène Kelch 13-propeller sur le chromosome 13. La stratégie de surveillance de la résistance aux médicaments à base d'artémisinine

implique une surveillance continue du biomarqueur Kelch 13-propeller pour détecter l'émergence de mutations qui pourraient annoncer une résistance aux médicaments dans la région. Dans cet article de revue narrative, nous avons examiné la littérature existante pour combler le manque de connaissances et accentuer l'importance de la surveillance de routine de la résistance à l'artémisinine en Afrique subsaharienne. Nous avons effectué notre recherche sur la base de données PubMed et Google Scholar pour identifier des articles, des rapports et des résumés évalués par des pairs sur la résistance aux médicaments à base d'artémisinine en utilisant les mots-clés suivants; «résistance aux médicaments à base d'artémisinine», «résistance aux médicaments antipaludiques», «thérapie combinée à base d'artémisinine», «Kelch 13-propeller», «gène K13-propeller» et «marqueur moléculaire K13». L'examen a fourni des informations pertinentes sur les dérivés de l'artémisinine, la polythérapie à base d'artémisinine, l'action moléculaire de l'artémisinine, la définition de la résistance à l'artémisinine, la base génétique de la résistance aux médicaments à base d'artémisinine et la découverte de Kelch 13, ainsi que l'importance de la surveillance de la résistance à l'artémisinine. La surveillance moléculaire peut fournir aux responsables des politiques de santé une prévision des menaces imminentes pour le traitement du paludisme. C'est d'autant plus vrai lorsque les médicaments sont en thérapie combinée, par exemple, la surveillance moléculaire peut donner un indice qu'un médicament échoue malgré le fait qu'en combinaison, il est toujours apparemment cliniquement efficace.

Mots clés: paludisme; artémisinine; résistance; marqueur moléculaire; gène Kelch à 13 hélices

Introduction:

Malaria, caused by *Plasmodium falciparum*, places immense burden on resource-poor countries, particularly those from sub-Saharan Africa. Nigeria is known to contribute the highest burden to global malaria morbidity and mortality. The world recorded an estimated 227 million cases of malaria in 85 malaria endemic countries in 2019 (1). Malaria cases rose to 241 million in 2020, an additional increase of 14 million, from the previous year. Similarly, in 2020, there was an estimated malaria deaths of 627 million, a 12% (69, 000 deaths) increase from 2019 report. Majority of the 29 of the 85 endemic countries which accounted for the 96% of malaria cases and deaths are from sub-Saharan Africa (1). There is need for sustained, effective malaria control programme, to stem the tide of morbidity and mortality arising from malaria, especially in all the 85 endemic countries.

Effective malaria control programme incorporates different kinds of malaria surveillance methods to its control programme scheme. Some of such malaria surveillance schemes include, active case detection for case investigation (reactive), active case detection of febrile cases at community level (pro-active), mass screening, uncomplicated *P. falciparum* cases routinely admitted, and case reporting from private sector. Outside these surveillance schemes, there is also need to monitor susceptibility of the mosquito vector to the common insecticides and the response of the *Plasmodium* spp to the current antimalarial drugs in use.

Molecular markers associated with antimalarial drug resistance are being used to monitor and map the extent of spread of the resistance and hence plan effectively for necessary containment programmes. Molecular markers are excellent tools for surveillance of antimalarial drug resistance. They have been useful as predictors of emerging or existing levels of

antimalarial drug resistance in many malarial endemic countries. Three of the molecular markers often used to monitor resistance to ACTs include the *P. falciparum* multidrug resistance 1 (PFMDR1), *P. falciparum* Ca²⁺-ATPase (PfATP6) and Kelch-13 propeller domain (PfK13) marker.

Perhaps, if there had been an effective surveillance on the spread of chloroquine and sulfadoxine/pyrimethamine resistance to other malaria endemic nations early enough, followed by containment programmes, the global resistance spread experienced with those erstwhile first-line drugs would have been averted. In Nigeria, chloroquine and sulfadoxine/pyrimethamine antimalarial drugs were used as first-line of drugs for treatment of malaria. However, the result of the 2002 and 2004 antimalarial efficacy studies showed that chloroquine and sulfadoxine/pyrimethamine were no longer adequate for national first line use (2). The attendant rising cases of treatment failures with these drugs, strengthened by the recommendation of the World Health Organization (WHO), led to the Federal Ministry of Health in Nigeria proscribing their use as first-line for treatment of malaria. The need to move from monotherapy antimalarial regime to more effective combination therapy rose, and with further efficacy trials in 2004, two artemisinin-based combination therapies were recognized. Nigeria, thus moved from the use as first-line, the failing chloroquine and sulphadoxine/pyrimethamine antimalarial drugs to artemisinin combination therapy (ACT) in 2005 (2).

There has been considerable progress in combating the malaria burden globally. Mortality rates arising from malaria was estimated to have declined by 62% globally between 2000 and 2015 and by 29% between 2010 and 2015. Seventeen countries eliminated malaria (attained zero indigenous cases for 3 years or more) (3). In sub-Saharan African countries, the number of people infected with malaria parasites has

declined from 131 million in 2010 to 114 million in 2015 (3). All these were made possible by adherence to WHO-recommended intervention policies and strategies: vector control, larval control, intermediate preventive therapy (IPT) in infants, children and pregnant women, improved and accessible diagnostic tools, and prompt treatment of cases.

The introduction of highly effective ACTs in 2005 by the WHO, particularly added to the gains (4). However, the gains made so far, seem to be threatened by the recent emergence in South East Asia (Cambodia, Myanmar, Thailand, Vietnam, and Laos) of *P. falciparum* resistant to ACTs (5,6,7). South East Asia has been noted as the centre hub for previous development and subsequent spread of *P. falciparum* strains resistant to erstwhile effective and potent antimalarial drugs.

Artemisinin derivatives:

Artemisinin was discovered in China from medicinal plant, *Artemisia annua*, locally known as Qinghao (8). The discovery of artemisinin as an antimalarial drug is tied to the urgent military need that came during the Vietnam War in 1960s and 1970s. The war witnessed huge loss of military personnel on both warring sides. To assuage further loss and deal with the effect of chloroquine-resistant *P. falciparum*, the Chinese government responded to the request of the North Vietnamese by researching to develop new antimalarial drugs. It was this research that led to the discovery of artemisinin (8,9).

At the Academy of Chinese Medical Sciences in Beijing, a team of researchers found that wormwood extract was 100% effective against rodent malaria, although the outcome was inconsistent. The inconsistency in result was later discovered to be due to the use of the traditional method of extraction which was destroying the pharmacologic constituent of the extracts. This understanding paved way for the development of an alternative extraction method of using cool ether. The problem of inadvertently damaging the pharmacologic constituent was solved and with further researches, they were able to purify the effective component and determine the structure to be an endoperoxide located within a sesquiterpene backbone (10,11).

Artemisinin is a sesquiterpene lactone, and since its initial discovery, pharmacologists/pharmacists have tried to modify the original structure at C10 position to produce more potent derivatives such as artesunate, artemether, artemotil, arteether, and dihydroartemisinin (DHA). Originally, artemisinin and its derivatives were used to treat malaria, however activities against cancer (12), parasites such as *Toxoplas-*

ma (13), *Clonorchis* (14), *Schistosoma* (15), *Leishmania* (16), and viruses (17) have recently been documented. The artemisinin prototype and its derivatives possess important pharmacological qualities that heighten their antimalarial activity such as rapid onset of action, short half-life and activity against different stages in the life cycle of the malaria parasite (18). They are also known to kill the sexual stages (gametocytes) of malaria parasites in the circulation (19).

Artemisinin-based combination therapy (ACT): What it is!

The idea of combining an artemisinin with another structurally unrelated antimalarial drug was to optimize malaria treatment. This was seen as the best therapeutic strategy to improve treatment efficacy and slow down the development of resistance to the individual drugs in the combination (20,21). The multidrug concept of treating infectious diseases has been applied in the treatment of human immunodeficiency virus (HIV) infection and tuberculosis. It is believed that the combination therapies which involve the use of drugs with different targets and mechanism of action, decreases the chance of emergence of drug resistance.

The treatment success achieved from combination therapy is underpinned by the assumption that drug resistance depends on DNA mutation. The assumption maintains that provided that the constituent drugs in the combination have different modes of action, the probability of a parasite developing resistance to both drugs simultaneously will be highly reduced compared to developing resistance to one drug (22). Most artemisinins combined treatments contain one of the three artemisinin derivatives including artemether, artesunate or dihydroartemisinin (DHA). Blood esterases and hepatic cytochrome P450 enzymes respectively convert oral artesunate and artemether to DHA, but they are active themselves (23). Although artemisinins have short elimination half-life of about 1 hour, the time is ample for maximal effects against the different life-cycle stages of the malaria parasites. However, to make-up for the rapid elimination of the active metabolite (DHA) from the blood stream, the administration of artemisinin with long-acting agents is required (24).

Two prominent pharmacodynamic properties of artemisinin determine how best they are used. One is their ability to kill both ring stage and more mature trophozoites rapidly, a property that underlie their importance in life-saving efficacy in severe disease and in cure (25). The other property is that patients treated with artemisinin always have a subpopulation of

artemisinin-treated ring-stage parasites enter a state of dormancy, avoiding being killed outrightly. This subpopulation of parasites resume growth a few days or weeks after stoppage of therapy (26). This is seen as the reason for the approximately 10% failure rates observed with the use of artemisinin monotherapy in malaria treatment (27). It was for this phenomenon that artemisinins are best used in combinations with a long-acting partner drug. In this way, the artemisinin derivative kills the most of the parasites within a few days, while the few remaining parasites are killed by the long-acting partner drug (28).

Five of the combined formulations currently recommended by the WHO for the treatment of uncomplicated *P. falciparum* malaria are; artesunate-amodiaquine, artesunate-mefloquine, artesunate-sulfadoxine/pyrimethamine, dihydroartemisinin-piperaquine and artemether-lumefantrine (29). Artesunate-amodiaquine, artesunate-mefloquine and artemether-lumefantrine fixed-dose ACTs have been implemented as first-line treatment for uncomplicated malaria in most malaria endemic countries (30). Piperaquine was originally added as partner drug to ACTs for the first-line treatment of uncomplicated confirmed-malaria cases in Cambodia, Vietnam, Myanmar and China (31). A new ACT recently introduced into the market is artesunate-pyronaridine (32). The WHO has recommended ACTs since 2005, and treatment with these drugs provide excellent cure rates, above the minimum acceptable threshold of 90% (23).

Molecular action of artemisinin:

The knowledge of exactly how artemisinin and its derivatives work at the molecular and cellular level to bring about the cellcidal effect has been elusive for a while. Several models from *in vitro* medicinal chemical studies have tried to explain this at one or the other, however, it seems consensus has not been reached yet. A known basic feature of artemisinin is that they are unstable in the presence of alkaline or acidic milieu, and they react in the presence of peroxide with certain reducing agents such as Fe^{2+} , Cu^{2+} and haeme. The endoperoxide bridge has been shown to be critical to artemisinin's antimalarial and anti-cancer properties. The pharmaceutical properties of artemisinin are thought to be associated with their reductions, though the mechanisms that bring about this reduction is still uncertain (33,34).

Four models considered to be possible modes of action of artemisinin include; haeme-detoxification pathway interference, induction of alkylation of translationally controlled tumour protein, interference with mitochondrial func-

tion, and inhibition of the sarco/endoplasmic reticulum membrane calcium transporting ATPase 6. A study by Sun et al., (35), using the baker's yeast (*Saccharomyces cerevisiae*) demonstrated the molecular and cellular properties of artemisinin. In the model, they proposed that the anti-mitochondrial and haeme-mediated reactive oxygen species (ROS)-generating properties constitute two cellcidal actions of artemisinin.

Definition of artemisinin resistance:

Adopting a global consensus definition of artemisinin resistance has been a big challenge. However, a proposal was made some times ago that a clinical case of artemisinin resistance would have the following attributes (36); (i) persistence of parasites on the 7th day after the start of treatment or re-emergence of parasites within 28 days after the start of treatment; (ii) adequate plasma concentrations of dihydroartemisinin, a major artemisinin metabolite; (iii) prolonged parasite clearance time; and (iv) reduced *in vitro* susceptibility of the parasite.

A study carried out at different sites in Western Cambodia in 2007-2008 reported a significant delay in parasite clearance time following treatment with artesunate monotherapy and with an ACT, when compared with that conducted in eastern Thailand (37). In any patient from South Asia with uncomplicated *P. falciparum* malaria, and a starting parasite count of 10,000 parasites/ μl of blood, artemisinin resistance is defined as a parasite clearance half-life 5 hours following treatment with an artesunate monotherapy or an ACT (38). In the study done by Witkowski et al., (39) in 2013, they defined artemisinin resistance in *P. falciparum* as a parasite survival rate 1% in the ring-stage survival assay ($\text{RSA}^{0-3\text{h}}$) *in vitro*. The assay involved adapting clinical parasite isolates to culture, and synchronizing them at the early-ring stage (0-3 hr post-invasion of red blood cells), exposing them to a pharmacologically-relevant dose of DHA for 6 hours and then culturing for 66 hours further. The survival rates were assessed by counting the proportion of viable parasites that developed into second-generation rings or trophozoites with normal morphology at 66 hours ($\text{RSA}^{0-3\text{h}}$), after drug removal.

Genetic basis of artemisinin drug resistance and discovery of Kelch 13:

The slow parasites clearance rates experienced with administration of artemisinin prompted many genotype-phenotype research works that were aimed at unravelling the phenomenon. A region on *P. falciparum* chromosome 13 was found to be strongly associated with slow clearance of the parasites in genome-wide stu-

dies (40). The major breakthrough in the search of genetic basis of the reduced susceptibility of the *P. falciparum* parasites to artemisinin came in 2013 when parasites subjected to artemisinin pressure, were cultured for 5 years and then sequenced (41). A mutation was found close to the chromosome 13 region in the genome association studies (40,42). Arley et al, (41) by comparing the whole-genome sequences of drug-selected and unselected parasite lines, identified on chromosome 13, a single-nucleotide polymorphism (SNP) in the *PF3D7_1343700* gene that encodes a M476I substitution in the propeller domain of a kelch protein. This kelch protein which contains a 'kelch' motif is now generally being referred to as K13.

The use of K13-propeller polymorphism as a molecular marker of artemisinin resistance in Cambodia was validated after demonstrating that 17 different K13 mutations were present in parasites from this country and that the predominant C580Y mutation had increased in prevalence especially in areas where artemisinin resistance was so common. Also, that the common mutations; C580Y, R539T, and Y493H were associated with prolonged clearance half-lives and elevated RSA^{0-3h} survival rates (43). Many more researchers have since conducted more studies establishing the fact that K13-propeller polymorphism is a marker for artemisinin resistance

even in other Great Mekong Regions in South-east Asian countries including Thailand, Vietnam, China and Myanmar, with the K13-propeller mutation findings being also associated with slow parasite clearance (44,45).

In Africa, some studies have reported dozens of K13-propeller mutations, many of which are different from those found in SE Asia (46-48). These African variants of mutations are still at low frequency, and are yet to be associated with artemisinin resistance in patients and *in vitro* in 17 countries (49-51). However, more recently, validated Pfk13-propeller mutations have been reported in India, South America, and on the African continent, in Uganda and Rwanda (52-57). Also in Nigeria, V692G, B664I, Q661H, and C469C mutations detected in a study by Fehintola et al., (58) were consistent with delayed parasitological clearance. Few other studies conducted in Nigeria, demonstrated presence of Pfk13 propeller gene mutations, however, they could not be linked to ACT failure or delayed clearance (59,60).

Different Pfk13 mutations produce varying effects on the clearance phenotype. Box 1 below shows the WHO list of candidate/associated and validated markers of artemisinin partial resistance and the criteria for the classification. The list of candidate and validated markers is updated continually (Table 1).

Box 1: WHO list of candidate/associated and validated markers of artemisinin partial resistance and the criteria for classification (61)

Candidate or associated Pfk13 markers of artemisinin partial resistance	A statistically significant association ($p < 0.05$) between a Pfk13 mutation and clearance half-life > 5 hours or day 3 parasitaemia via a chi-squared test or appropriate multivariable regression model on a sample of at least 20 clinical cases OR Survival of $> 1\%$ using the RSA0-3h in at least five individual isolates with a given mutation or a statistically significant difference ($p < 0.05$) in the RSA0-3h assay between culture-adapted recombinant isogenic parasite lines, produced using transfection and gene editing techniques, which express a variant allele of Pfk13 as compared with the wild-type allele
Validated Pfk13 markers of artemisinin partial resistance	Both requirements 1 and 2 are met

Table 1: Pfk13-propeller gene markers of artemisinin partial resistance (61)

Validated markers		Candidate or associated markers	
F446I	P574L	P441L	
N458Y	C580Y	G449A	
C469Y	R622I	C469F	
M476I	A675V	A481V	
Y493H		R515K	
R539T		P527H	
I543T		N537I/D	
P553L		G538V	
R561H		V568G	

Why surveillance?

Artemisinin combination therapies are currently critical in the management of uncomplicated *P. falciparum* malaria in all sub-Saharan African nations, including Nigeria. No new drugs of equivalent efficacy have been developed to replace them and even if there are plans to produce more potent drugs, it will take some years. The emergence of artemisinin resistance in South East Asian countries is thus, a threat to the global malaria control and elimination action plans.

There is the premonition that artemisinin-resistant parasites will behave the same way they did with chloroquine and later pyrimethamine, spread westward to reach Africa. If this should happen, this would add to already high burden of malaria in this region. Emergence of chloroquine resistance was estimated to have caused the death of millions African children (62). This trend should not be allowed to repeat itself. There is therefore need for a surveillance scheme to be put in place in order to monitor the emergence and spread of artemisinin-resistance to other malaria endemic regions which should prompt drastic containment measures.

Conclusion:

The emergence and subsequent spread of antimalarial drug resistance has been one of the main challenges to malaria containment in many malarial-endemic areas such as sub-Saharan African countries. Detection and characterization of resistance to chloroquine and sulfadoxine/pyrimethamine that were once used as first-line in the treatment of malaria occurred after resistance had already spread globally, hence there was no opportunity for containment.

With the emergence of artemisinin drug resistance in Southeast Asia, and in line with the WHO Global Plan for Artemisinin Resistance Containment (GPARC), there is need to proactively avert the spread of artemisinin resistance to sub-Saharan Africa by constantly monitoring its spread and instituting containment measures.

Contributions of authors:

AAI and GIO conceptualized this study. AAI, EM, and PAO conducted the literature searches, collated articles, and drafted this paper. PCE and GEN, and ABO reviewed, edited, and contributed to the final version of the manuscript.

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Conflict of interest:

Authors declare no conflict of interest.

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