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Resistance to Artemisinins in Africa and the WHO Reservations About use of A. annua in Africa

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Abstract

Artemisinin resistance was first identified in Cambodia in 2008. In the Mekong region, once artemisinin resistance has spread widely, it is often followed by resistance to its associated drugs, leading to failure of combination therapy. This resistance is associated with parasites carrying genetic mutations. Despite a denial of resistance to artemisinin and other antimalarials in Africa by the World Health Organization and other institutions such as the Pasteur Institute, health professionals are still alerted to this resistance.

In this article, we present a non-exhaustive literature on the reports of resistance to Artemisinin and other antimalarials in Africa.

The researchers point out that the emergence of partial artemisinin resistance in Africa is an alarm of a great public health danger, if these resistance to related drugs spread rapidly in Africa, the effectiveness of treatment could be compromised. Recent data from Africa suggest that we are on the verge of clinically significant artemisinin resistance.

That African policy makers and researchers reflect on alternative malaria treatments in Africa. We need to accelerate research on medicinal plants including Artemisia annua and afra in Africa.

Keywords

ACTs, Alu, K13 , ASAQ, WHO,

1. Introduction

According to the WHO World Malaria Report (2021), there were approximately 241 million malaria cases and 627,000 malaria deaths worldwide in 2020. This represents approximately 14 million more cases in 2020 than in 2019 and 69,000 more deaths. About two-thirds of these additional deaths (47,000) were related to

disruptions in the delivery of malaria prevention, diagnosis, and treatment during the during the pandemic ***(1).***

The report shows that Africa has the highest malaria burden in the world. 95% of cases and 96% of deaths are concentrated in sub-Saharan Africa, and 80% of malaria malaria deaths in Africa occur in children under five years of age (2).

One of the major challenges in the fight against malaria that the scientific community has to face is the great ability of P. falciparum to develop resistance mechanisms against the molecules that are submitted to it. Indeed, over the years, P. falciparum has become resistant to almost all the antimalarial drugs that have been used **[3].**

In order to contain and prevent this growing phenomenon of chemoresistance, the WHO recommends the use of drug combinations, one of the molecules of which should be artemisinin (or derivatives) [4].

To this end, the DRC adopted in 2005, like most endemic countries, the use of artemisinin-based combination therapies (ACTs) for the first-line treatment of malaria. To date, two combinations have been validated by the Ministry of Health and are used: Artesunate-Amodiaquine (ASAQ) and Artemether-Lumefantrine (ALu).

Artemisinin, a molecule that allows rapid clearance of parasites from the bloodstream, has begun to lose its effectiveness, as have the other antimalarial molecules used previously. To date, this resistance remains confined to Southeast Asia, more precisely to the Greater Mekong region [5-6]. All experts agree that the spread of this resistance to the African region would be catastrophic.

The discovery of a molecular marker for artemisinin resistance in 2004 by Ariey et al [7] offers the possibility to periodically monitor the emergence of artemisinin resistance in Africa. Numerous studies have been carried out to explore the presence or appearance of artemisinin-resistant mutant strains in some African countries, but fortunately none of the "Asian" mutations conferring resistance have been found [7-8].

Artemisinin resistance in P. falciparum is asso- ciated with pfkelch13 polymorphisms encoding the parasite's Kelch 13 (K13) propeller domain, which consequently serve as a molecular marker in sur- veillance (9)

In a study of Edwin and all , the prevalence of K13-propeller mutations in sub-Saharan Africa was described on samples collected in several countries . This baseline information will be essential for monitoring the emergence and/or spread of P. falciparum artemisinin resistance in sub-Saharan Africa [10].

This study observed an urgent and important need for local studies of clinical artemisinin resistance and in vitro and ex vivo RSA0-3 hour data to clarify the significance of K13 helix mutations as markers of artemisinin resistance Africa. [10].

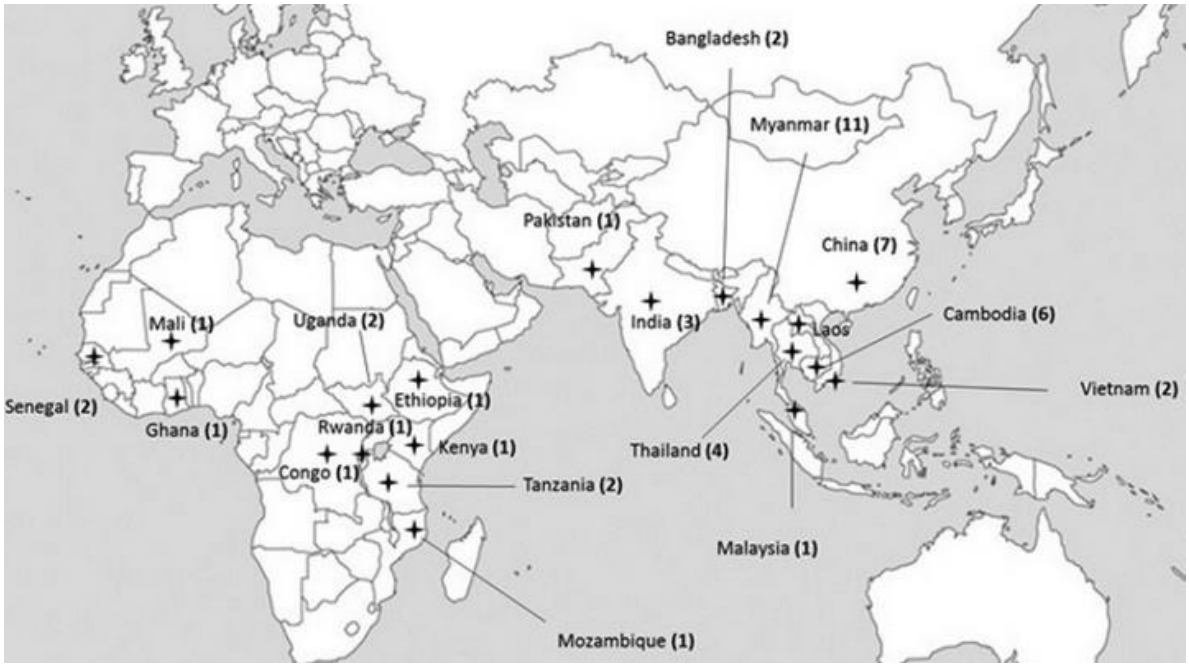


Fig.1. Map of worlwide artemisinin resistance (Kelch13 mutations) (11)

Republic Democratic of Congo, DRC Resistance

A systematic review paper from RDCongo found 5 resistance genes related to ACT tratment.

The review of Mvumbi et al , articles were classified based on year of collecting, year of publication, sample size and characteristics, molecular markers analysed and polymorphisms detected.
Some of them concerned non-Congolese individuals but supposedly infected in DRC. Five genes were analyzed in these studies: the Plasmodium falciparum chloroquine resistance transporter gene (pfcrt), the dihydropteroate synthase gene (pfdhps), the dihydrofolate reductase gene (pfdhfr), the Plasmodium falciparum multidrug resistance 1 gene (pfmdr1), and the K13 propellant gene (k13) (12).

Uganda Resistance

Several reports from African health professionals confirm this dramatic situation of antimalarial ineffectiveness.

The Kiguba (Uganda) article confirms that one in five health professionals reported suspected or confirmed therapeutic ineffectiveness of ACT treatment to at least one competent authority in the previous six months, whist is significantly higher than the documented extent of adverse event reporting by health professionals in the same setting . (13)
In Uganda an independent selection was identified of three polymorphisms in the pfmdr1 gene following administration of AL in a region of Africa where malaria is highly endemic. These polymorphisms were not associated with clinical treatment failure but are evidence for the ability of this drug combination to drive selection of parasites toward resistant phenotypes. These polymorphisms were not associated with clinical treatment failure, but provide evidence of the ability of this drug combination to drive parasite selection towards resistant phenotypes. (14)

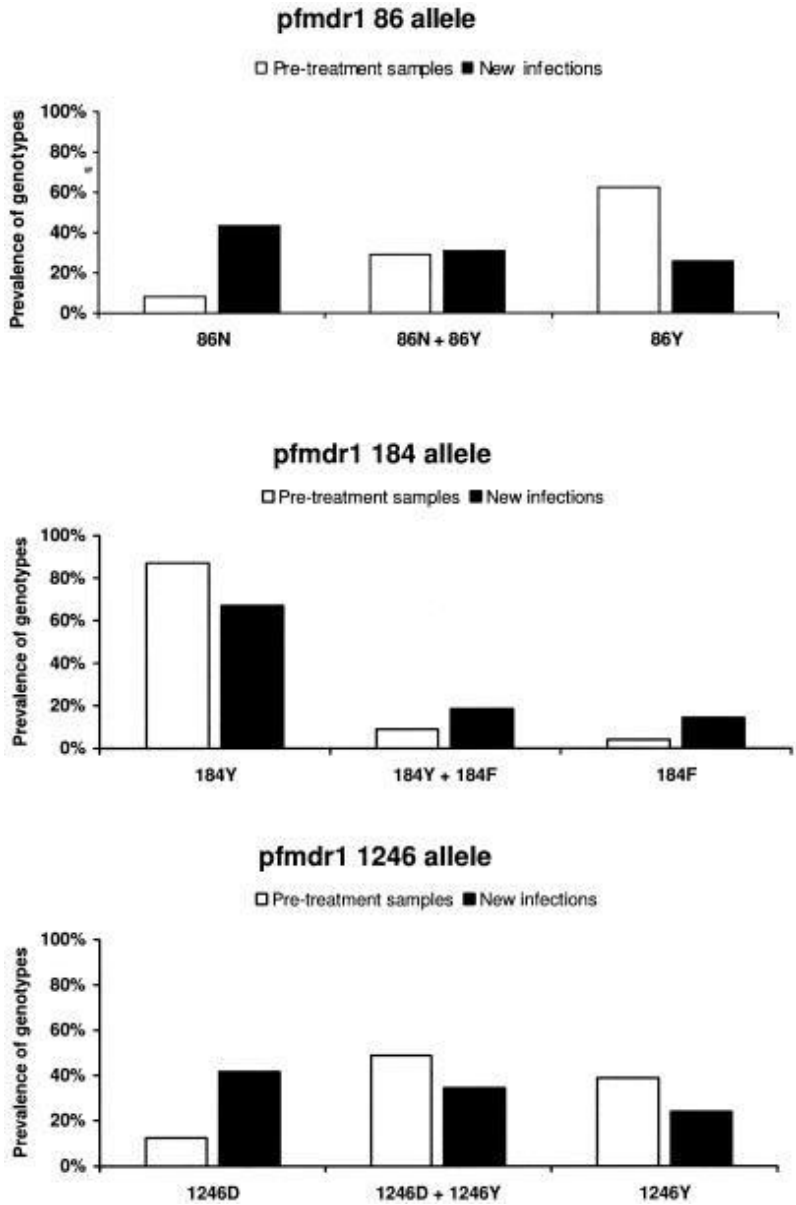


Fig.2. Prevalence of *pfmdr1* alleles in pretreatment samples and samples from newly infected patients following therapy with artemether-lumefantrine. Alleles typically classified as wild type are on the left, mixed infections are in the middle, and those classified as mutant are on the right.

In Uganda Plasmodium genotypes with decreased sensitivity to artemether-lumefantrine increased from 2008 to 2012 in a study involving 312 children. Another trial reports failures in artemether-lumefantrine treatment (15 -16).

Another clinical trial with artemether-lumefantrine in Uganda gave catastrophic results: Late parasitological failure 137 (32.9%), Late clinical failure 74 (17.8%) 7 (2.0%). Adequate clinical and parasitological response 189 (45.4%) (17).

Nigeria Resistance

At the top of the list of the most affected countries, Nigeria accounted for 26.8% of patients in 2020. The country also has the highest mortality rate with 31.9%. This is more than double that of the Democratic Republic of Congo (13.2%), the second most affected nation by this disease, to which children under five and pregnant women are very vulnerable.

A study Monday Tola , describes mutations in Plasmodium falciparum genes associated with drug resistance in malaria; Pfk13, Pfmdr1, PfATPase6 and Pfcrt in isolates obtained from 83 symptomatic malaria patients collected in August 2014, aged 1-61 years in southwest Nigeria.
In Nigeria they find that some Pfmdr variants are present at a prevalence of 56%. (18)

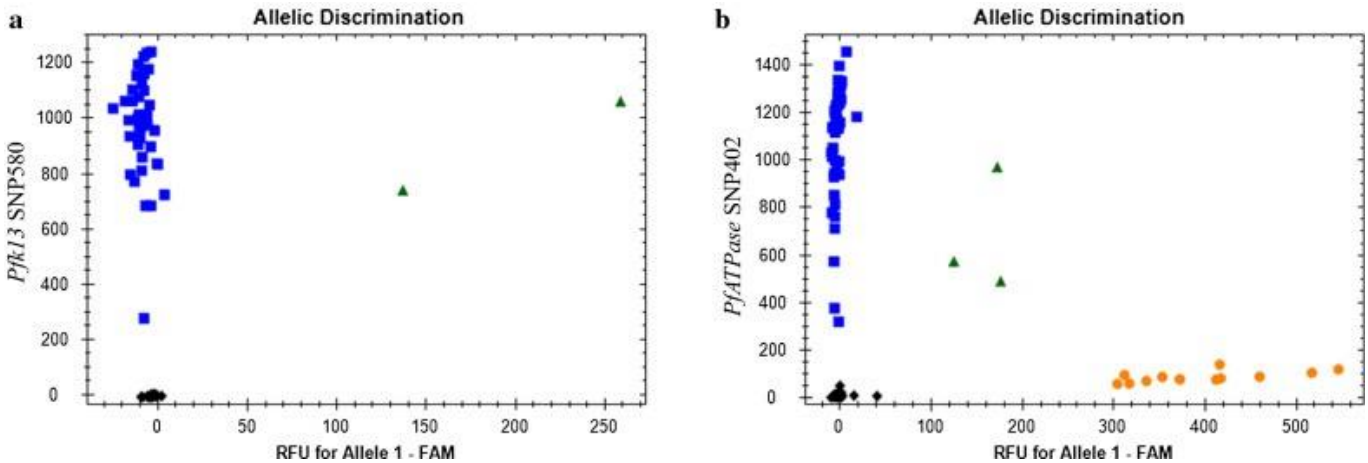


Fig.3. Allelic discrimination of wild and mutant genes in parasite samples (a) *Pfk13* SNP580 and (b) *PfATPase* SNP402. DNA from dried blood spots (DBS) were extracted and used for TaqMan allele discrimination assays. Blue points depict wild type alleles, green for mixed allele calls and orange for mutant variants. Untyped reactions are shown in black.

In Nigeria a study reveals a need to re-evaluate the quality and efficacy of artemisinin-based combination therapy agents in Nigeria and Sub-Saharan Africa. Though six ACT combination therapies are available, but malaria is resistant to one of the longer-acting drugs and patients had bad reactions to another, so only four ACTs are recommended. Christian Happi, a malaria of Redeemer's University in Lagos declares that among thousands of blood samples anlyzed 80–90% have at least one mutation. (19,20,21,22)

Angola Resistance

In a study in Angola, The results of the pfcrt and pfmdr1 sequence analyses were consistent with the literature showing an overrepresentation of the 76T pfcrt allele in amodiaquine treatment failures and a predominance of the N86 pfmdr1 allele in AL treatment failures and confirms the high prevalence of the N86 allele in the circulating parasite population in Angola and the high prevalence of the N86 allele even in the ASAQ treatment failures in this study. Notably, the fixation or near fixation of the N86 allele, as well as clinical evidence of reduced efficacy, may have implications for the future of LA use in Angola. (23)

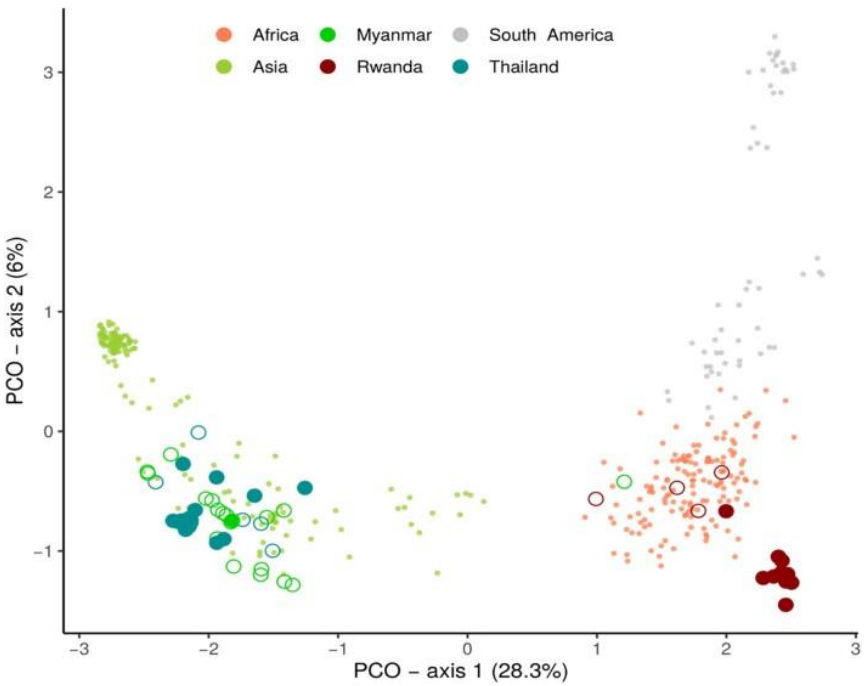
In Angola resistance to lumefantrine and artemisinine derivatives was extensively studied. (24)

But some French experts refuse to recognize these facts. If an African medical doctor publishes a scientific paper describing the shorthcomings of ACTs, their reaction is violent and obnoquious. They even ask the scientific journal to retract the published paper.

Finally, after so much evidence accumulated in many African countries, WHO and Pasteur also recognize that ACT resistance spreads in Africa, in this case Rwanda. Idem a German team.

Rwanda Resistance

In this Rwandan genomics study, Rwandan Pfkelch13 561H mutants were phylogenetically related to other African samples and clustered unambiguously with Rwandan Pfkelch13 WT parasites. Haplotype analysis revealed that the Rwandan Pfkelch13 561H mutants shared an identical haplotype surrounding the R561H mutation that differed from the haplotypes of the SEA 561H mutants, strongly suggesting a unique de novo epidemiological origin and recent spread of the mutation. But which had no genetic relationship to the Pfkelch13 561H mutants detected in Myanmar and Thailand.(25)



Principal Coordinate Analysis (PCoA) based on pairwise genetic distances in a 494 kb window around the *Pfkelch13* gene.

Fig. 4. ***Principal Coordinate Analysis including* Pfkelch13 *wild type and 561H isolates including those sourced from a public database (small dots, the MalariaGEN* Plasmodium falciparum *Community Project, https://www.malariagen.net/apps/pf/4.0)and originating from different continents (Asia, Africa or South America). Isolates originating from populations where the* Pfkelch13 *R561H mutation was found are emphasized (large dots). Empty large dots correspond to* Pfkelch13 *wild-type isolates and filled large dots correspond to* Pfkelch13 *561H mutants. While the mutants tend to cluster with individuals of similar origin, axis 1 clearly discriminates African (Rwanda) from Asian (Thailand and Myanmar)* Pfkelch13 *561H mutants.***

Another study from Rwanda confirm the presence of K13 mutations are Rwanda and that their prevalence in P. falciparum malaria patients in Huye district has increased from 0% in 2010 to >12% in 2019. The validated artemisinin resistance mutation R561H is present in 4.5% of P. falciparum isolates transmitted in this region. The emergence of artemisinin resistance mutations in Rwanda is alarming as it may indicate the development of resistance to commonly used antimalarial drugs in this region. (26)

Tanzania Resistance

Also, in the neigboring country, Tanzania, known drug resistance mutations were seen at increased frequency in northern districts

In a Tanzanian study that separated northern and southern districts and identified genetically mixed populations in the north. Isolates from nearby districts were more likely to be genetically related than parasites collected from more distant districts. Known drug resistance mutations were observed at increased frequency in northern districts (including two infections carrying pfk13-R561H), and additional variants of undetermined significance for antimalarial drug resistance also varied by geography.(27)

In Tanzania the overall prevalence of NFD haplotype claimed to be associated with emerging artemether-lumefantrine tolerance ranges from 17 to 26% among other haplotypes. With continuation of ALu as first-line drug and in the absence of CQ and AQ, this haplotype is expected to keep rising. There is need for continued pharmacovigilance studies in order to predict early parasite tolerance to the drug.(28)

In Tanzania the temporal selection of molecular markers associated with artemether-lumefantrine tolerance/resistance may represent an early warning sign of impaired future drug efficacy. This calls for stringent surveillance of artemether-lumefantrine efficacy and emphasizes the importance of molecular surveillance as a complement to standard in vivo trials.(29)

In Tanzania the difference between individual treatment groups and the next best treatment combination was significant ($p<0.001$) in every case. Recrudescence rates by day 28, after correction by genotyping, were 48.4%, 34.5%, 11.2%, and 2.8%, respectively. The study shows how few the options are for treating malaria where there is already a high level of resistance to sulfadoxine-pyrimethamine and amodiaquine. (30)

In Tanzania a study suggested that drug pressure selection for increased parasite virulence and infectiousness may be occuring in human populations in Africa.(31,32)

In a recent study in Tanzania a wide range of pfk13 transcript variation was observed throughout all timepoints after artemether-lumefantrine treatment. The findings suggest that a reduced PfK13 transcriptional response may represent a first step towards artemisinin tolerance/resistance. (33)

Over the last century all monotherapies (quinine, chloroquine, mefloquine, lumefantrine, piperaquine, pyrimethamine, halofantrine) have led to rapid resistances of *Plasmodium falciparum.* Combination therapy between artemisinin and molecules with long lasting action had raised optimism. But already in 2003 first signs of resistance developed in South-East Asia. It has been established meanwhile that they were mostly related to mutations in the kelch13 propeller region of the parasite. Mutations have meanwhile raised to 90%. (34)

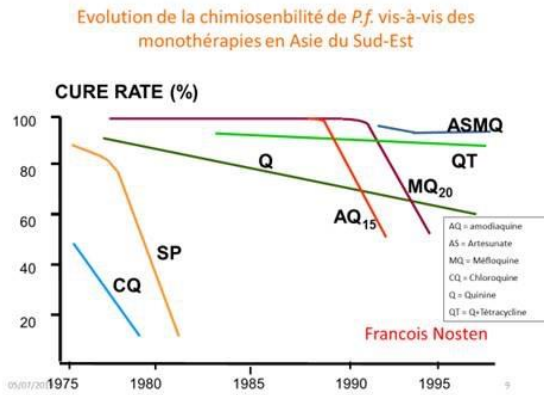


Fig.5. Evolution de la Chimionsebilité P.f.

Artemisinin resistance in *Plasmodium falciparum* has emerged in South-East Asia, has spread over several countries and now poses a threat to the control and elimination of malaria.

But the problem is not limited to South-East Asia. Signs of arteminisin resistance have developed in other continents, in at least a dozen African countries.

Researchers from the London School of Hygiene and Tropical Medicine have discovered a new genetic mutation in *Plasmodium falciparum*, the parasite that causes malaria, which may mean the A recent review identified emergence of potential ART-resistance mediating k13 mutations in the African region. Diversity of mutations in pfkelch13 gene is high in African region.

Kenya Resistance

In Kenya authors conclude that parasite clearance time after artemisinin-based combination therapy (ACT) may be increasing in Asian and African settings.(35)

On the Kenyan coast the significant, albeit small, decline through time of parasitological response rates to treatment with ACTs may be due to the emergence of parasites with reduced drug sensitivity,(36)

In Kenya the findings of another research team call for close monitoring of parasite genotypic, phenotypic and clinical dynamics in response to current first-line treatment in western Kenya. Having been the first focus of chloroquine resistance in Africa western Kenya will be crucial in informing the next steps on the deployment of first-line treatment of uncomplicated malaria in the possible future era of attenuated response of artemisinin.(37)

In a malaria endemic area in Kenya a K13 propeller sequence analysis of *P. falciparum* parasites Kenya did not detect the predicted artemisinin-resistant genotypes, but some temporal substitutions were observed(38, 39)

Impact of pre-existing immunity on artemisinin combination therapy (ACT) efficacy was examined in Kenya to monitor resistance, and for implementation of new treatment strategies. The number of individuals with lag phase was significantly higher in the Artemether-Lumefantrine compared to the Artesunate-Amodiaquine (40)

Mali Resistance

In Mali K13-propeller mutations were identified in both recent samples and pre-ACT infections.

In Mali a study by Ouattara concluded that K13-propeller mutations can occur at a low frequency, independent of drug selection by artemisinin treatment or possibly, selection pressure by other drugs (e.g., chloroquine). K13-propellant proteins have been associated with reduction and oxidation stress management (REDOX) of the cell,16,17 a type of effect characteristic of the action of most antimalarial drugs on P. falciparum, including chloroquine.18 The management of stress or the effect of other drugs in the selection of K13-propellant mutations and the relevance, if any, of these K13-propellant mutations on artemisinin efficacy will require further investigation, including genetic transformation studies(41)

Senegal Resistance

In Senegal the increased prevalence of Pfmdr1 duplication in P. falciparum isolates from patients in Dakar within a 2-year period is cause for concern and vigilance (42)

Sudan Resistance

In Sudan the findings of a study call for a need to review the Sudan malaria treatment policy. Epidemiological factors could play a major role in the emergence of drug-resistant malaria in eastern Sudan.

In this study a total of 371 pre-treatment samples were analyzed for molecular markers of MS resistance. Temporal changes and geographic differences in the frequency distribution of MS resistance genotypes showed evidence of regional differentiation and selection of resistant strains. The results of this study call for the need to review the malaria treatment policy in Sudan. Epidemiological factors may play a major role in the emergence of drug-resistant malaria in eastern Sudan.(43)

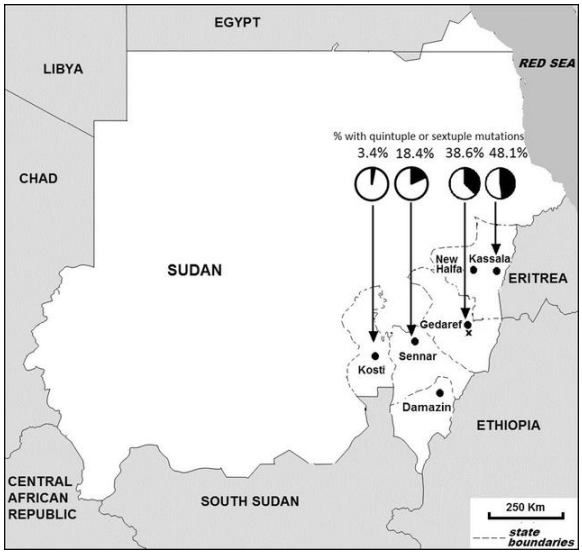


Fig 6: High efficacy of artemether-lumefantrine and declining efficacy of artesunate + sulfadoxine-pyrimethamine against *Plasmodium falciparum* in Sudan (2010–2015): evidence from in vivo and molecular marker studies

Liberia Resistance

In Liberia it was found that although treatment is highly efficacious, selection of molecular markers in reinfections could indicate a decreased sensitivity or tolerance of parasites to the current treatments and the baseline prevalence of molecular markers should be closely monitored (44).

Ghana Resistance

In Ghana the persistent detection of low density Plasmodium sp. Infections, following antimalarial treatment suggests these may be a hitherto unrecognised obstacle to malaria elimination. The presence of variants of the validated markers of artemisinin resistance as well as persisting polymorphisms after 14 years of artemisinin-based combination therapy use argues for continuous surveillance of the markers. In another study in Ghana a high prevalence of ASAQ resistant parasites was already noticed in 2008.(45,46.47)

Ethiopia Resistance

In Ethiopia high rates of recurrent parasitemia were noted for AL and CQ against *Plasmodium vivax* less frequently against *Plasmodium falciparum (48,49)*

Somalia Resistance

In Somalia a failing first-line treatment (AS + SP), with a failure rate above the threshold (10%) for policy change, and a high prevalence of quintuple mutations were found; (50)

Mozambique Resistance

In Mozambique, after the decrease in clinical malaria incidence observed until 2009, a steady resurgence of cases per year has been reported nationally, reaching alarming levels in 2014.(51)

Gabon Resistance

In Gabon severe artemisin resistance was noticed.(52). In Gabon another study confirms these intriguing results. The strongest correlation between diminished DHA sensitivity and MF resistance was observed, followed by correlation between diminished DHA sensitivity and CQ resistance. Cross-resistance between CQ and MF was also observed.(53)

In Gabon a study shows an increase in the prevalence of childhood plasmodial infection and a high frequency of markers associated with AL treatment failure.(54)

Burkina Faso Resistance

In Burkina Faso clinical trial NCT00808951 shows a higher occurrence of recurrent malaria infections during a 28-day follow up period for artemether-lumefantrine. Already at the MIM 2009 conference at Nairobi it was stated that 3 years after the ACT introduction there are concerns about the decrease level of theses ACTs efficacy. The risk of treatment failure was already 29,2 % for artemether-lumefantrine.(55)

In a recent trial in Burkina Faso there is a lowering of the cure rate after ACT treatment and around 50% of the treated patients develop a new malaria episode within 28 days

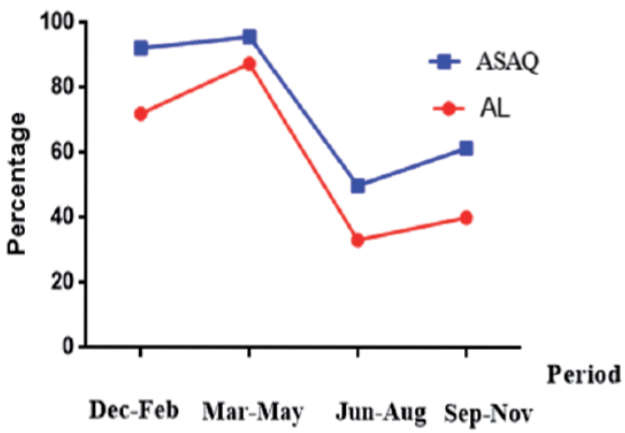


Fig 6. Temporal variation of PCR uncorrected Adequate Clinical and Parasitological Response by treatment group(56)

Artemisia annua and the WHO reservations use in Africa

Artemisinin, the central molecule of the current antimalarial strategy, is produced from the leaves of the annual mugwort or Artemisia Annua. This plant has been widely used for almost two millennia in the Chinese pharmacopoeia *[57]*. The use of ACTs recommended by the WHO is nevertheless subject to certain constraints constraints specific to artemisinin such as: the counterfeiting of antimalarial molecules molecules marketed in Africa [58-59], the long duration for cultivation, extraction, processing and manufacturing of the final product, the complexity of manufacturing operations and

quality control, but also the relatively high relatively high cost [60]. Many studies have been conducted to assess the appropriateness of this practice and opinions are so far very mixed in the scientific community.

On the one hand, Diawara HZ et al. report an efficiency rate > 95% of herbal tea d'Artemisia annua L. sur P. falciparum [61]; Munyangi et al. conclude in their study that infusions based on A. annua and A. afra would confer better results that the combination ASAQ [62]. Weathers et al. on the other hand argue for the inclusion of A. annua dried leaf tablets in the arsenal antimalarial therapy [63].

On the other hand, the results of Mueller et al. which describe high rates of recrudescence after use of herbal teas based on A. annua, thus advise against the use of A. annua as an alternative to antimalarial drugs [64]; the WHO also gives its position stating that it does not recommend the use A. annua in all its forms for the treatment or prevention of malaria [65]; in France, the National Agency for Drug Safety (ANSM) suspended the placing on the market of a product called Artemisia [66] and the National Academy of Medicine (ANM) has issued a statement warning health authorities and populations of malaria transmission areas on recommendations scientifically uncertain and irresponsible for the use of this herbal medicine" [67. An online survey conducted by Malaria World (a platform of experts in the field) yields 32% vs 68% respectively for people favorable to the use of herbal teas based on A. annua and for those who are there oppose [68].

One of the main reasons cited by those who advise against the use of herbal teas based on A. annua is the probability of emergence of strains resistant to artemisinin following continuous low-dose administration.

However, to date, no scientific work has shown a clear correlation A. annua herbal tea and resistance to artemisinin.

Conclusion and Suggestions

In this paper, we have attempted to present a current assessment of our knowledge of resistance to artemisinin and its derivatives in Plasmodium falciparum in Africa, with emphasis on molecular studies and factors affecting its distribution in endemic populations.

It remains surprising that, despite the studies on these phenomena of antimalarial drug resistance, African governments do not disclose any research to seek alternative solutions to the current treatments of malaria.

We suggest and encourage the acceleration of research on African medicinal plants including clinical research on Artemisia in Africa.

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