Acta Parasitologica (2022) 67:55–60
https://doi.org/10.1007/s11686-021-00489-y

REVIEW

Potential Emergence of *Plasmodium* Resistance to Artemisinin Induced by the Use of *Artemisia annua* for Malaria and COVID-19 Prevention in Sub-African Region

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Received: 28 June 2021 / Accepted: 26 October 2021 / Published online: 19 November 2021

Abstract

Plasmodium resistance to antimalarial drugs is an obstacle to the elimination of malaria in endemic areas. This situation is particularly dramatic for Africa, which accounts for nearly 92% of malaria cases worldwide. Drug pressure has been identified as a key factor in the emergence of antimalarial drug resistance. Indeed, this pressure is favoured by several factors, including the use of counterfeit forms of antimalarials, inadequate prescription controls, poor adherence to treatment regimens, dosing errors, and the increasing use of other forms of unapproved antimalarials. This resistance has led to the replacement of chloroquine (CQ) by artemisinin-based combination therapies (ACTs) which are likely to become ineffective in the coming years due to the uncontrolled use of *Artemisia annua* in the sub-Saharan African region for malaria prevention and COVID-19. The use of *Artemisia annua* for the prevention of malaria and COVID-19 could be an important factor in the emergence of resistance to Artemisinin-based combination therapies.

Keywords COVID-19 · Malaria · Resistance · Artemisinin · *Plasmodium falciparum*

Background

Malaria is caused by parasites of the *Plasmodium* species and it is a global public health burden. In 2019, WHO reported 228 million malaria cases and 405,000 deaths worldwide, with a predominance in sub-Saharan Africa [1]. In December 2019, new pneumonia called coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), with some clinical features similar to malaria such as fever, was reported in Wuhan, China [2]. COVID-19 rapidly evolved into a global pandemic, declared by the World Health Organization (WHO) on March 11, 2020, as a Public Health Emergency International Concern (PHEIC) [3]. At September 30, 2021, there were more than 234,390,731 confirmed cases in 223 countries and deaths exceeded 4,792,848 [4].

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The use of plants for health care is a matter of culture and tradition in Africa [5–9]. It should be noted that for primary health needs, a large part of the African population resorts to traditional medicine, whose remedies mostly come from plant origin [6, 10]. The preference for these remedies is due to their accessibility and their low cost [11, 12]. This use has increased with the emergence of COVID-19. Thus, to prevent this disease, people deliberately use extracts of *Artemisia annua* (a plant containing artemisinin), which is also used by people to prevent malaria [13, 14]. Malaria is endemic in tropical and subtropical low-income countries, and the inadequate use of antimalarial drugs, especially artemisinin, for malaria prevention and in the context of COVID-19 could lead to an increase of *Plasmodium* resistance to antimalarial drugs, including artemisinin and its derivatives, thus compromising their efficacy. Therefore, such resistance would affect malaria control in these regions and jeopardize efforts to eliminate malaria by 2030 [15]. Our review focused on the selection factors of *Plasmodium* resistance to antimalarial drugs and the risk of emergence of resistance to artemisinin and its derivatives due to the empirical use of *Artemisia annua* in one part, and in another part the need to monitor regularly the efficacy of antimalarial drugs to achieve the goal of malaria elimination in Africa on time.

Selection Factors for *Plasmodium* Resistance to Antimalarial Drugs

Drug pressure has been identified as a key factor in the emergence of antimalarial drug resistance [16]. Antimalarial drug resistance must be considered in two parts: on the one hand, the initial genetic event that produces the resistant mutant, and on the other hand, the subsequent selection process in which the survival advantage in the presence of the antimalarial drug leads to the preferential transmission and spread of resistance [17]. Indeed, the evolution of antimalarial drug resistance is facilitated by several factors, including the use of counterfeit forms of antimalarial drugs, inadequate controls on prescribing, poor adherence to treatment regimens, incorrect dosing, and the increasing use of other forms of unlicensed antimalarial drugs [18, 19]. This last factor has attracted more attention from WHO in recent years with the deliberate use of *Artemisia annua* extracts for malaria prevention and COVID-19 [20–23].

About artemisinin, while this plant is currently cultivated in various regions of African countries, studies have revealed that there is a diversity of *Artemisia annua* species, and for the same species, the artemisinin content can vary from one region to another depending on the composition of the soils on which these plants are grown [24, 25]. Thus, these Artemisia extracts are likely to exert undesirable drug pressure over a long period of time once their concentrations fall below the critical threshold and may select for resistant parasites [26].

In Africa, the resistance to CQ had led to its replacement by artemisinin-based combination therapies (ACTs) [27–29]. This resistance has appeared in Southeast Asia, for all classes of antimalarial drugs and recently for artemisinin, the main component of current antimalarial drugs [16, 30, 31] with an increase in markers of this resistance in African countries [30]. Not only the effects of resistance on morbidity and mortality are generally underestimated [32, 33], but it is also an obstacle to malaria elimination [34, 35].

Resistance of *Plasmodium* to Artemisinin and Its Derivatives

Artemisinin has been discovered since the early 1970s by Dr. Youyou Tu, the 2015 Nobel Laureate, as an effective drug for the treatment of malaria [36]. Following WHO recommendations, artemisinin-based combination therapies (ACTs) are used for malaria treatment in Africa, because fast-acting artemisinin can immediately reduce parasitaemia, allowing the remaining parasites to be eliminated with a long-acting partner drug [37]. Although recent studies have confirmed the existence of artemisinin resistance in *P. falciparum* [38], artemisinin and its derivatives have nonetheless made progress in the treatment of malaria and have made quinolones as a secondary treatment option in most countries of the world [38, 39].

Since 2014, mutations in the "helix" region of a *P. falciparum* Kelch protein (encoded by the kelch13 gene) have been identified as molecular markers of artemisinin resistance based on their association with the slow clearance phenotype [40].

In Asia, 36.5% of K13 mutations were distributed in two areas, one in Cambodia, Vietnam and Laos, and the other in western Thailand, Myanmar and China [41].

In Africa, non-synonymous mutations are still rare and very diverse. Non-synonymous K13 mutations have been reported in Angola, Burkina Faso, Cameroon, Central African Republic, Comoros, Congo, Ivory Coast, Democratic Republic of Congo, Equatorial Guinea, Ethiopia, Gabon, Gambia, Kenya, Liberia, Madagascar, Malawi, Mali, Mozambique, Niger, Nigeria, Rwanda, Senegal, Sierra Leone, Togo, Uganda and Zambia [42]. However, a recent study in Uganda found an association between K13 mutations and delayed parasite clearance in subjects treated with artesunate, a soluble arteminisin derivative [30].

In Togo, 1.8% of K13 mutations were identified in three sentinel sites monitoring antimalarial efficacy in 2013 [40]. Although *K13* helix mutations associated with artemisinin resistance have been found in Tanzania and Uganda [30, 41],

there is no evidence of emerging resistance to artemisinin and its derivatives associated with K13 mutations in Togo in particular [40].

Furthermore, immunity may also play an important role in the emergence and transmission potential of artemisinin-resistant parasites [43]. Most malaria infections are genetically diverse [44–47], and this diversity increases in areas of high transmission where hosts experience frequent and overlapping infections [48–51]. In a multi-infected host, different lineages compete for the same resources while being subject to specific and non-specific immune regulation. When drug-resistant and drug-sensitive parasites are present in the same host, the sensitive lineages can suppress the growth and transmission of the resistant lineages [52–54]. However, reduced malaria transmission decreases naturally acquired immunity, which may influence the emergence of artemisinin-resistant phenotypes and genotypes of *Plasmodium falciparum* over time [55]. It is important to understand how changing transmission and immunity could impact the emergence of artemisinin resistance, especially as increased malaria control and elimination activities may improve the immunological conditions for the expansion of artemisinin-resistant *P. falciparum* [56].

To ensure that the current COVID-19 pandemic does not result in a major upsurge in malaria cases, derailing sub-Saharan Africa elimination efforts, it is critical that the recommended malaria case management practices and procedures are closely monitored [57].

Use of Domestic Artemisinin for the Treatment of COVID-19

The effective antimalarial drugs recommended by the WHO are made from artemisinin isolated from *Artemisia annua* [58]. Thus, populations especially in malaria-endemic areas empirically use infusions of *Artemisia annua* leaves to prevent or treat malaria [59]. While its active ingredient (artemisinin) content is dependent on geo-climatic factors and season [60], it is currently planted in several countries for domestic use against malaria [61].

With the emergence of COVID-19 pandemic, many populations have illegally adopted *Artemisia annua* for the prevention and treatment of COVID-19, despite WHO calls against its use without proper scientific approval [20–23]. For example, Madagascar has used a tonic (Covid Organic) from *Artemisia annua* as a potential remedy for COVID-19 [62]. Unfortunately, no studies to date have elucidated the interactions of artemisinin on the angiotensin-converting enzyme 2 (ACE2) receptor, which is known to be the critical cellular binding receptor for SARS-CoV-2 [63], although studies have demonstrated the anti-inflammatory potential of high-dose artesunate in the context of COVID-19 [64, 65]. This may partially justify the rejection of some institutions such as WHO to allow artemisinin as a cure for COVID-19 [23].

Impact of *Plasmodium* Resistance to Antimalarial Drugs on the Achievement of the New Malaria Strategy Plan

Remarkable advances in malaria control in recent years have been partially wiped out by the COVID-19 pandemic [66]. Therefore, WHO recommends attention to malaria interventions while responding to the pandemic to avoid the unintended consequences of SARS-CoV-2 on malaria in Africa [3]. Although medicinal plants such as *Artemisia annua* are considered as possible treatments for COVID-19, its use has been discouraged by the WHO, due to lack of data on the efficacy of the extracts and lack of safety information [67–69]. In fact, its use could potentiate the development of resistance to ACTs, especially in Africa where the malaria burden remains the highest [1].

However, resistance has been shown to occur primarily during the ring stage of parasite development due to multiple forms of mutations in the Kelch PF3D7_1343700 (K13-propeller) helix domain on chromosome 13 [70]. Mutations in the K13-propeller lead to an increase in phosphatidylinositol-3-kinase (PfPI3K), which is required to mediate cell signalling and survival [71]. The latter leads to vesicle expansion that increases engagement in the unfolded protein response (UPR) [72]. Vesicle expansion (rather than increasing individual genetic determinants of UPR) effectively induces artemisinin resistance, presumably by promoting "proteostasis" (protein translation coupled with proper protein folding and vesicle remodelling) to mitigate artemisinin-induced proteinopathy (death due to abnormal overall protein toxicity) [73]. This may jeopardize malaria elimination plans in African countries and other malaria-prone regions, as artemisinin monotherapy and especially low-dose artemisinin monotherapy could lead to the spreading of the *Kelch13*-positive strains in Africa [74]. While resistance to most malaria drugs (amodiaquine, lumefantrine, mefloquine and sulfadoxine–pyrimethamine) has already been well demonstrated [74, 75], the uncontrolled use of artemisinin-based drugs could be a factor for the emergence of resistance in the coming years [76]. Following the pattern of CQ resistance, *Plasmodium* may become increasingly resistant to artemisinin in the coming years.

Furthermore, although control strategies for COVID-19 have been significantly improved by vaccination [77, 78], malaria control strategies are disrupted, particularly in low-resource settings where clinical facilities are extremely limited [66]. Thus, controlling COVID-19 is a global challenge. Malaria elimination would be effective if efforts against

COVID-19 were also engaged in the fight against malaria, which causes more than 400,000 deaths per year in Africa, with children being the most affected [1, 79–81]. Given that the burden of malaria is highest in low-income tropical countries that have little capacity to fund malaria control and elimination programmes, malaria control in these regions is likely to be hampered in a few years by the spreading of the *Kelch13*-positive strains. To verify this hypothesis and to trace this potential risk, it would be good to set up regular monitoring sites in all African countries to detect in time any suspicious increase of molecular markers or its appearance, so as to be able to act quickly to prevent its propagation to other regions of the world.

Conclusion

Plasmodium resistance to antimalarial drugs is an obstacle in the fight against malaria. The resistance to ACTs especially could be potentiated by the use of *Artemisia annua* for the prevention and treatment of malaria and COVID-19. Although COVID-19 has a collateral impact in Africa, this pandemic could be an important factor in the emergence of TCA resistance. Therefore, more concerted efforts are needed to defeat these two diseases.

Author Contributions EA, AMD and CTN contributed to the design of the study and analysed the research data. TB, TT, KDK, KY and TA provided additional articles related to the topic. EA, AMD and CTN wrote the review which was then read and approved by all the other authors.

Declarations

Conflict of Interest The authors declare that they have no competing interests.

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