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Narrative Review of Artemisinin Resistance Mechanisms

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ABSTRACT

Artemisinin and its derivatives are the cornerstone of global malaria treatment, primarily through artemisinin-based combination therapies (ACTs). These drugs have significantly reduced malaria morbidity and mortality due to their rapid parasite clearance and broad efficacy. However, the emergence of artemisinin resistance, first detected in Southeast Asia, now poses a critical threat to malaria elimination efforts. Resistance is characterized clinically by delayed parasite clearance and is strongly associated with mutations in the Plasmodium falciparum kelch13 (PfK13) gene. Beyond PfK13, multiple genetic loci, biochemical adaptations, and physiological mechanisms contribute to parasite survival under drug pressure. This review synthesizes current knowledge of resistance mechanisms, geographical spread, clinical implications, and strategies to mitigate the threat. Effective containment will require continued molecular surveillance, novel therapeutic development, and integrated malaria control strategies.

Keywords: Artemisinin resistance; Plasmodium falciparum; PfK13 mutations; Antimalarial drugs; Malaria epidemiology; and Drug resistance mechanisms.

INTRODUCTION

Artemisinin, a sesquiterpene lactone endoperoxide, is the foundation for frontline treatment of Plasmodium falciparum malaria. Allies of the World Health Organization in the Greater Mekong Subregion partnered in 2000 to eliminate the four species of Plasmodium present in the region by 2030 [1]. Emergence of artemisinin resistance will likely jeopardise the frame for elimination and threaten malaria control progress worldwide. Resistance to artemisinin derivatives (artesunate, artemether, dihydroartemisinin) with a clear geographic pattern has emerged in Cambodia and Thailand; foci appeared in the Gambian population [2]. A mutation in the gene encoding the μ-chain of the AP2 adaptor complex, a protein involved in endocytosis and membrane protein trafficking, is associated with increased resistance to artemisinin in rodent malaria [1, 2]. Artemisinin is a sesquiterpene lactone extracted from the plant Artemisia annua, which has played a central role in treating malaria since the 1970s [1]. The plant extract was originally employed to prevent or treat fever in traditional Chinese medicine. The sequential development of derivatives such as dihydroartemisinin, artemether, and artesunate followed in the 1980s. The unique chemical structure comprises a highly reactive peroxide bridge essential for antimalarial activity [1]. This mediator rapidly interacts with reduced heme iron (Fe^{2+}) produced during haemoglobin catabolism in the Plasmodium food vacuole [3]. The reaction generates carbon-centred free radicals capable of alkylating haem groups, proteins, lipids, and DNA, causing widespread parasite damage.

Artemisinin reduces parasitaemia by more than 10,000-fold within 48 hours [4]. The global deployment of artemisinin-based combination therapies (ACTs) has thus become a cornerstone of malaria treatment. However, parasites harbouring PfK13 mutations survive the initial artemisinin challenge, restricting clearance and threatening progress towards malaria elimination [13, 18]. Artemisinin resistance consequently remains the greatest concern for communicable disease control worldwide.

Malaria: A Global Health Challenge

Malaria is a major global public health challenge that is caused by infection with Plasmodium parasites, transmitted by mosquitoes [3, 15]. The most severe infections and deaths are caused by infections with P.

falciparum and Plasmodium vivax. The World Health Organization (WHO) estimated that, in 2016, there were 216 million malaria cases and 445,000 deaths worldwide [5]. A large majority of infections and deaths occurred in sub-Saharan Africa. It is a deadly disease primarily afflicting pregnant women and children less than 5 years old. Artemisinin combination therapy (ACT) is at present the most effective treatment to control and/or eliminate malaria. However, artemisinin resistance threatens to reverse these gains. Artemisinin was introduced in the early 1990s. Early-resistance foci to artemisinin in the assays in Southeast Asia threaten current treatments [6]. Before the introduction of artemisinin, treatment was significantly hindered as a consequence of increasing global resistance of parasites to inexpensive antimalarial drugs, including chloroquine, sulfadoxine-pyrimethamine, and mefloquine. Malaria is a major global public health challenge caused by infection with Plasmodium parasites, transmitted by mosquitoes [6, 7]. The majority of infections and deaths are caused by P. falciparum and Plasmodium vivax. Malaria remains a major burden, with 216 million cases and 445,000 deaths in 2016, mainly caused by P. falciparum. Resistance to antimalarial agents, especially artemisinins, threatens to reverse these gains and threatens global control efforts [5]. Malaria is a deadly disease primarily afflicting pregnant women and children less than five years old [6, 7]. Artemisinin combination therapy (ACT) is currently the most effective treatment to control and eliminate malaria infection. Oxidative stress caused by exposure to artemisinins does not mount an adaptive stress response or protection against other sources of oxidative stress. Subsets of molecular pathways of resistance to artemisinins have been characterized. The well-characterized marker of resistance is the K13 polymorphism, and additional K13-independent mechanisms of resistance have been described[3, 9]. Models based on metabolism, stress pathways, and cell cycle indicate important determinants of quiescence induction and recovery underlying an essential phenotype in artemisinin resistance and elucidate various aspects of Plasmodium biology [5, 18].

Mechanisms of Drug Resistance

Drug resistance occurs when parasites can survive and multiply despite antimalarial drugs at concentrations that would normally eliminate them [2, 17]. The development of resistance by pathogens and parasites constrains the therapeutics available to the medical community and remains a constant threat to human health [3, 15]. There are numerous possible ways a pathogen may evade a chemotherapeutic agent, from excluding the drug through efflux pumps, to enzymatic degradation or chemical modification of the drug, to modification of the targets, to changes in cellular physiology that counteract potential damage or extend damage repair mechanisms [3, 18]. Highly adaptive in nature, resistance often arises through a series of stepwise mutations, which individually offer a selective survival advantage that eventually accumulates and leads to an outright resistant genotype [4]. Drug resistance manifests both clinically as treatment failures and in vitro as increased IC50, the drug concentration that kills 50 % of the parasites in culture [5]. Drug resistance can be classified as either true or delayed. True drug resistance occurs when the parasites survive throughout the entire treatment regime and eventually cause a recrudescence, that is, a return of symptoms. Delayed drug resistance is a less clear phenomenon and is generally determined by slower clearance of parasites during the treatment course of the drug, though if clearance is slow enough, delayed resistance may be considered a recapitulation of true resistance. With Plasmodium falciparum, resistance to potent antimalarials such as chloroquine and sulphadoxine-pyrimethamine, which have long in the past been considered a part of first-line therapies, has been recorded in all endemic malaria regions [1]. Resistance to the current standard of care, artemisinin combination therapies (ACTs), has also emerged, further threatening the progress made in controlling this deadly disease.

Definition and Importance

Drug resistance is the ability of a microorganism to resist the effects of antimicrobial drugs to which it was once susceptible [4]. Resistance can be classified as either primary resistance in patients with no prior drug exposure or secondary resistance arising because of previous exposure to the drug [4]. Secondary resistance may be further subdivided into three types: (1) acquired resistance, in which the pathogen has mutated or acquired genetic material allowing resistance; (2) bypass resistance, in which a drug-activated pathway that normally inhibits cell growth is blocked by a secondary mechanism; and (3) intrinsic resistance, in which a pathogen is naturally resistant to a drug because the drug lacks the properties required for activity against that pathogen [4].

Types of Drug Resistance

Resistance describes a phenomenon where parasites survive and grow despite standard drug treatment that would normally be effective [1]. It may be innate (natural, preexisting) or acquired after drug–parasite interaction. Resistance is classified as cross, multi-, or pleiotropic [3]. Cross-resistance implies that an increase in tolerance to one drug results in tolerance to a related molecule; multi-resistance signifies resistance to two or more chemically unrelated drugs. If resistance to one drug results in simultaneous increased susceptibility for others, these latter phenotypes are known as pleiotropic [1, 3].

Artemisinin Mode of Action

Artemisinin is a sesquiterpene endoperoxide derived from Artemisia annua that displays fast-acting activity and high efficacy against chloroquine-resistant Plasmodium falciparum parasites [7]. The key biochemical step in the antimalarial activity of artemisinin and its derivatives involves the cleavage of the endoperoxide bridge. Iron-mediated cleavage of the peroxide bridge generates reactive species such as carbon-centered free radicals and reactive oxygen species (ROS) that attack multiple essential parasite proteins and other cellular targets, leading to parasite death [5]. The Endoperoxide Bridge is essential for artemisinin's antimalarial efficacy, and modifications that disrupt this moiety lead to complete abrogation of antimalarial activity. In the intraerythrocytic phase, cleavage of the Endoperoxide Bridge is mediated by ferrous iron derived from the digestion of haemoglobin in the parasite food vacuole or by the ferrous haem moiety itself [5, 7]. Artemisinin molecules alkylate a large array of parasite proteins, including those involved in proteasomal degradation, glycolysis, the tricarboxylic acid cycle, purine metabolism, and those residing in multiple subcellular organelles, including the food vacuole, mitochondria, apicoplast, cytosol, and the endoplasmic reticulum [2, 5]. In asexual blood stages, artemisinin targets both the trophozoite and ring stages, although trophozoites are killed at much lower doses, presumably because of their higher digestive activity of haemoglobin and hence an increased intraparasite supply of ferrous iron or haem molecules. Rings, by contrast, are less susceptible, and micromolar concentrations of the drug are needed to clear them. In gametocytes, artemisinin shows stage specificity with the highest activity observed during the early stages I-III and no activity against mature stage V forms [5, 6].

Biochemical Mechanisms

Drug resistance in malaria can be categorized into molecular, biochemical, and physiological types, offering additional perspectives [5, 9]. Biochemical resistance in Plasmodium falciparum is evident when the drug's mode of action is weakened or abolished. There may be significant chemical alterations during the drug's metabolism, or the mechanism of drug activation may be impeded. Disrupting the primary effect of a drug removes the killing or inhibiting activity on the parasite [4, 18]. Two main biochemical aspects involved in artemisinin resistance are the antioxidant defense used by malaria parasites to combat reactive oxygen species (ROS) and the ability to reprogram their metabolic activities and growth[6, 17]. Recently, it has been suggested that artemisinin-resistant parasites can reprogram metabolic processes to evade the drug's killing effect. Their heightened sensitivity to proteasome inhibitors, coupled with the association of proteasome genes with the resistance phenotype, implies that proteostasis is pivotal for survival in the presence of artemisinin. While the exact origin of proteotoxic stress remains unclear, oxidative stress generated upon drug activation, damage to parasite proteins, and a growth retardation phenotype characteristic of resistant parasites may collectively contribute to heightened dependence on proteostasis mechanisms [5, 18].

Cellular Targets

Artemisinins, a group of sesquiterpene endoperoxides derived from Artemisia annua, represent the most important antimalarial drugs in current use. Acting rapidly and consequently short-lived in the human circulation, the main role of artemisinins in ACTs remains to ensure rapid parasite clearance from the bloodstream, reducing the chance of continued transmission [5]. Because of their rapid clearance, artemisinins exert most of their effects within the parasite bloodstream stages and also act against gametocyte formation, preventing onward transmission to mosquitoes. Although widely used as first-line antimalarials to treat the symptoms of malaria, the cellular targets and mode of action of artemisinins remain ill defined, and only recently have molecular markers for artemisinin resistance been identified [8]. The discovery of the cellular targets and mode of action of artemisinins is important for understanding the molecular basis of artemisinin resistance [5, 8].

Emergence of Resistance

Since 2006, there has been growing concern over the ability of the parasite to become partially resistant to the effects of artemisinin, and the breakthrough discovery in 2008 of the highly artemisinin-resistant parasite from Western Cambodia prompted further investigations [13]. Before this time, ACTs were considered an effective treatment, and it was believed that artemisinin resistance was unlikely because of the very fast action of the drug and its short half-life. However, a gradual reduction in susceptibility to artemisinin was detected in some areas in Myanmar, Vietnam, and the China-Myanmar border [15]. In addition, parasites resistant to both artemisinin and AQ were observed in the Cambodia-Thailand border area in 2013, raising concern that an artemisinin-resistant parasite could develop into a super-resistant strain, which is potentially more difficult to treat [3, 18]. Resistance to the previously utilised quinoline-based drugs, chloroquine and sulfadoxine–pyrimethamine, is known to have emerged in the same areas where artemisinin resistance is now found, and its spread was closely monitored by the WHO[3]. This has important implications because the history of drug resistance has demonstrated that malaria parasites can develop cross-resistance to unrelated drugs [14]. According to the WHO definition, confirmed

partial artemisinin resistance is said to have emerged when the parasites have clearance rates of less than 50% per 24 h or an increased clearance half-life in patients who received the WHO-recommended dose of an ACT regimen, whereas suspected partial artemisinin resistance has been documented when these resistance indicators are detected in the same geographical region but no PfK13 mutations have been observed [1, 17].

Geographical Distribution

Artemisinin resistance was first reported on the Thai-Cambodian border in 2008. Over the subsequent decade, resistance spread throughout Cambodia, then extended to Myanmar and southern Vietnam [1, 4]. In 2014, additional resistance foci were noted along the Thailand-Myanmar border. A distinct resistant parasite lineage appeared to have arisen independently in eastern Myanmar[5]. In 2019, delayed parasite clearance following artemisinin-based combination therapy treatment was observed in the Bangladesh-India border region. Resistance was also detected in Guyana, though it remains absent from sub-Saharan Africa [6, 9].

Epidemiological Trends

Artemisinin resistance was first observed in western Cambodia in 2006, subsequently emerging along the Thailand–Myanmar border by 2010 and then spreading broadly across mainland Southeast Asia [1]. Resistance distribution is discontinuous and near-uniform within a given region. Multiple subpopulations exist within continental Southeast Asia, with resistance alleles emerging independently in various endemic locales [7, 9].

Genetic Basis of Resistance

Mechanisms of drug resistance range from an increased von Mises stress on the membrane to biochemical alterations such as enhanced antioxidant defense [9, 11]. Resistance emerges globally with key genetic determinants found in the PfK13 protein. Parasite resistance can be defined as the ability to survive or tolerate drug concentrations beyond the normally susceptible threshold. Concepts include resistance, tolerance, persistence, and resilience, each describing specific patterns of survival and recovery following drug exposure [9]. The global incidence of P. falciparum malaria declined when artemisinin was introduced; however, the spread of resistance is counteracting the gains in malaria control [9]. The search for effective therapeutic approaches to counter resistance is ongoing. A major effort is directed towards understanding the molecular and cellular basis of resistance [10]. This background informs a comprehensive approach to the research efforts aimed at delineating resistance mechanisms in malaria [9, 10].

Mutations in the PfK13 Gene

Mutations in the PfK13 gene have been strongly linked to resistance against artemisinins, the most potent class of antimalarial drugs currently available [11]. The propeller region of the Plasmodium falciparum Kelch [13] protein shows particularly notable mutations, but the protein remains essential to normal asexual development [12]. Despite the molecular evidence for its role, the precise biological function of PfK13 has not been definitively resolved. Several PfK13 variants were identified in isolates from the China-Myanmar border, with the corresponding amino acid substitutions, F446I, N458Y, C469Y, and F495L subsequently characterized in the laboratory using isogenic parasite lines [12, 17]. Reversing the mutations to the wild-type allele attenuates resistance phenotypes for all cases except C469Y. The F446I substitution, which dominates in northern Myanmar, induces a prolonged ring stage without conferring heightened resistance at the laboratory threshold and entails minimal fitness cost [1, 7, 18]. By contrast, the N458Y mutation activates resistance pathways but imposes a severe fitness penalty, offering a rationale for its low abundance in natural populations. Screening with a PfK13 antibody reveals expression throughout the asexual cycle and in gametocytes. In ring-stage parasites, PfK13 localizes to multiple small punctate structures, approximately one to two per parasite, which partially colocalize with the endoplasmic reticulum [5, 8, 9].

Other Genetic Factors

Genetic polymorphisms in multiple loci besides falciparum kelch13 (PfK13) influence artemisinin susceptibility [9]. The D193Y variant of the parasite thioredoxin-like protein gene (pctl gene) emerged as a candidate molecular marker for surveillance. Other loci potentially involved include ferredoxin (fd), amino acid transporter [1], multidrug resistance protein (mdr2), and crt. Both fd and crt are linked to the metabolism of chloroquine and amodiaquine. Adaptations to artemisinin may involve a combination of variations in multiple loci acting synergistically or epistatically, complicating the establishment of definitive sets of predictors [7, 8]. Another candidate is Pfcoronin, a Plasmodium actin-binding protein that sequesters actin monomers [7]. Point mutations in the WD40 domain G50E, R100K, and E107V have been found in artemisinin-resistant field isolates from Senegal. Pfcoronin is highly expressed in the ring stage and localizes to the parasite cortex. Although Pfcoronin substantially modulates ring-stage survival under artemisinin contact, the corresponding mutant alleles do not confer in vitro resistance[13]. Resistance to anti-malarial drugs arises through mutations in parasites that compromise the efficacy of treatments such as artemisinin combination therapy (ACT). Polymorphisms and gene

amplifications associated with artemisinin resistance remain ambiguous, lacking clear correlations to resistance phenotypes. Genome-wide analyses have identified chromosomal regions linked to slow parasite clearance rates; notably, a locus on chromosome [13] demonstrates a strong association, although specific genes remain unidentified. Experimental generation of resistant parasites has revealed genetic alterations like multidrug resistance gene [1] (mdr1) duplications, yet these phenotypes prove unstable in the absence of drug pressure [7].

Physiological Mechanisms

Reduced drug concentrations and extended cell survival are critical physiological mechanisms contributing to artemisinin resistance in Plasmodium falciparum[2, 12]. Modulation of cellular drug concentrations influences the parasite's exposure to the drug and determines the susceptibility thresholds attained under a specific dosage regimen [5]. Mutations at artemisinin interaction sites may reduce retention by affecting binding affinities or limiting access to cellular compartments, thereby lowering effective drug levels [5]. Endocytosis contributes to artemisinin transport, involving vesicular trafficking between organelles, and evidence indicates that modulating vesicular trafficking and endocytosis significantly impacts cellular drug concentrations [2, 8]. In artemisinin-resistant ring-stage parasites, hemoglobin endocytosis is reduced, resulting in diminished drug activation. Kelch13 (K13), located on cellular membranes at sites of endocytosis and coated pits, is among the earliest proteins to localize at vesicle formation sites, mediating vesicular trafficking between the plasma membrane and the early endosome. K13 mutations impair the formation of cytostome-delivering vesicles that internalize hemoglobin, reducing the cellular import of molecules facilitating artemisinin activation. Vesicles missing K13 accumulate in resistant parasites, amplifying vesicular and hemoglobin-trafficking defects [12, 15]. Kelch13 mutations also lead to the upregulation of unfolded protein response (UPR) pathways that mitigate artemisinin-induced damage. UPR mechanisms and ubiquitin/proteasome pathways are common cellular strategies to enhance proteostasis by confronting cellular stresses. Artemisinin exerts widespread cellular damage, and transmission-stage parasites containing K13 mutations co-opt these pathways to increase resistance [3, 13]. Although UPR pathways are initially downregulated during ring-stage development, K13 mutations accelerate the upregulation of UPR and proteostasis-related processes, enabling faster recovery from artemisinin exposure. Integration of vesicular transport and stress-response mechanisms elucidates how different resistance strategies collectively contribute to the resistant phenotype [5, 15].

Drug Transport and Efflux

Artemisinin resistance involves several concurrent physiological modifications that reduce the intracellular concentration of the drug and protect membrane phospholipids from peroxidative damage during haemoglobin catabolism[2]. Amino acid substitutions in PfCRT, which occur widely in parasite populations, alter the efficiency of drug transport by this carrier[12, 13]. Transporters that mediate the subsequent export of xenobiotics from cells, including the P-glycoprotein homologue PfMDR1, exhibit high rates of polymorphism in different regions of malaria endemicity, and can either amplify or compensate for the effects of PfCRT mutations on drug sensitivity [13]. Several other components that participate in cytostomal endocytosis and haemoglobin uptake, and regulate the metabolic state of the parasite during the transition between dormant and proliferative growth phases, are also required to deplete artemisinin concentration and shield sensitive structures from oxidative stress[1].

Cellular Stress Responses

Cellular stress associated with artemisinin exposure may add another level of resistance, although the precise mode of action remains controversial [2]. Experimental evidence, complemented by omics studies, points toward a role for the unfolded protein response (UPR) pathways in restoring homeostasis under the pressure of artemisinin-mediated alkylation. Activation of these pathways would allow the parasite to tolerate higher levels of artemisinin-induced damage and avoid entering dormancy, a growth-arrested state that enables survival during lethal drug concentrations [2, 8]. Notably, transcriptomic analyses have identified significant upregulation of genes implicated in the UPR and related stress response mechanisms in parasites exhibiting gene expression profiles associated with artemisinin resistance. The food vacuole of Plasmodium falciparum plays a central role in the regulation of cellular stress pathways for artemisinin[3, 17]. This specialized organelle is responsible for the catabolism of large quantities of host-derived hemoglobin, a critical process for parasite survival but one that generates reactive oxygen species via the Fenton reaction, which can induce protein damage and fragmentation. Physiologically, the parasite responds by activating the Kelch13-associated UPR. Collectively, these findings indicate that the Kelch13^C580Y mutation may modulate food vacuole activity, reducing proteotoxic stress and thereby enhancing artemisinin tolerance [2, 9].

Biochemical Adaptations

Antibiotic resistance arises either due to the modification of the amino acid sequence through mutation and/or due to the presence of an enzyme (newly acquired or already present in the body) that can modulate the available

antibiotic [1]. Biological adaptation is a fundamental principle of living organisms, from symbiosis to parasitism. Young parasites, therefore, can lay down the foundation for drug resistance mechanisms [3]. In response to artemisinins, P. falciparum enhances its antioxidant defense, possibly as a consequence of an enhanced unfolded protein response or increased haemoglobin digestion [1, 9]. From 0–3 hours post-invasion, the ring stage parasite undergoes metabolic quiescence with low levels of haemoglobin digestion and protein biosynthesis, mediated in part through reduced expression of ribosomal proteins. Shortly after ring-stage dormancy, Pfk13-mutant malaria parasites elevate the levels of PI3P vesicles [2, 17]. It is unclear whether the vesicles at the ER solicit the ring-stage quiescence. After the quiescence period, proliferation resumes with analog levels of haemoglobin digestion and a corresponding restoration of artemisinin susceptibility in trophozoites. Mutations in the transcription factor, PfAP2μ, the adaptor μ-chain of the AP-2 complex, are associated with increased survival of artemisinin exposure. AP-2 function is best characterized in other organisms as an essential component of the clathrin-mediated endocytic machinery. Neither AP-2 nor the previously implicated K13, however, interacts with clathrin in Plasmodium[4, 17]. The finding that a component of the AP-2 complex is involved in modulating artemisinin susceptibility is consistent with the notion that this key trafficking system contributes to K13-mediated artemisinin resistance. The proteasome ensures protein turnover, degrades damaged or toxic proteins, and regulates several vital cellular processes in Plasmodium[2]. Upon artemisinin treatment, the PfK13 mutant parasite reduces transcriptional activity to enhance unfolded protein response and proteasome-mediated degradation of damaged protein. Besides, an infection with either ACT resistance phenotype or ART resistance phenotype can reduce the therapeutic efficacy of artesunate–amodiaquine (ASAQ) and decrease the parasite clearance rate after artemisinin treatment, respectively[2, 9]. This artemisinin resistance COI pattern in parasite population highlights the challenge facing malaria treatment in the country; therefore, the efforts to limit resistance spread require urgent investigation to identify genes associated with multi-locus PAM site Single Nucleotide Polymorphisms (SNPs) as a basis for molecular marker identification [5, 9].

Antioxidant Defense Mechanisms

Malaria parasites exposed to artemisinin mount antioxidant defenses to reduce cellular damage from reactive oxygen species (ROS)[2]. Transition metals stimulate ROS production by catalyzing redox cycling, and Fe^{3+} is recycled to Fe^{2+} to potentiate ROS induced by antimalarial [3].

Metabolic Pathway Alterations

Several distinct metabolic pathway alterations accompany the quiescent state induced in resistant ring stages [12]. Artemisinin-resistant (ART-resistant) parasites exhibit a pronounced reduction in pathways that normally generate the free heme essential for artemisinin activation [3, 5]. Reduced haemoglobin (Hb) catabolism and a rerouting of carbohydrate metabolism, presumed to limit proteotoxic damage and growth, appear central to resistance [5]. Furthermore, PfK13 mutations reprogram the parasite's response to nutrient limitation, decoupling developmental progression from starvation and leading to decreased DNA replication and protein translation. PfK13 abundance determines the extent of these metabolic adjustments and correlates with the degree of ART resistance observed for individual mutations. PfK13 mutant parasites also exhibit diminished mitochondrial activity and altered interactions between the mitochondrion and the apicoplast that indirectly affect metabolic homeostasis and cellular signaling[5, 12]. While specific PfK13-dependent processes overall promote parasite survival, the precise mechanisms remain unresolved [5].

Impact of Resistance on Treatment Outcomes

The emergence and spread of ART resistance, followed by the widespread appearance of resistance to partner drugs in artemisinin combination therapies (ACTs), pose a serious threat to the management of malaria in endemic regions[2, 5]. In Western Cambodia and Thailand, clinical isolates of Plasmodium falciparum exhibited resistance to chloroquine, mefloquine, quinine, and the antifolates used as partner drugs in ACTs. The basis of this resistance was thoroughly documented and linked with specific gene mutations [2, 18]. For example, resistance to mefloquine has been associated with the increased number of copies and mutations of the P. falciparum multidrug resistance gene 1 (Pfmdr1), resistance to quinine has been linked to mutations in the P. falciparum multi-resistance protein 1 gene (Pfmrp1), and high resistance to pyrimethamine has been associated with amino acid substitutions in P. falciparum dihydrofolate reductase (Pfdhfr)[1, 18]. The emergence and spread of resistance to the partner drugs in the ACT combination have significantly worsened clinical outcomes, resulting in the loss of effectiveness of ACT against falciparum malaria in Cambodia and in parts of northern Myanmar and northeastern Thailand, where ACT failure rates have been reported to reach as high as 40%. Genetic mutations in Pfcrt, Pfmdr1, Pfmrp1, Pfdhps, and Pfdhfr have all been associated with decreased susceptibility to partner drugs such as amodiaquine, mefloquine, quinine, pyrimethamine, and sulfadoxine. Consequently, to control the spread of resistance, current

treatment policies now recommend close monitoring of the efficacy of the drugs in use and the implementation of new treatment combinations [2, 3].

Clinical Implications

Artemisinin-based combination therapies (ACTs) rank among the most effective treatment regimens against P. falciparum malaria [1, 3]. Nevertheless, the rise and global spread of artemisinin resistance threaten to culminate in additions to the population at risk of severe morbidity and mortality. Resistance has already reached critical levels in South-East Asia, and according to recent indications and prediction models could emerge soon in other regions of the world [4, 9]. Drug resistance generally increases parasite load during infection and may jeopardize the efficacy of available treatment regimens, ultimately leading to the diminution or loss of the effect of once highly efficient drugs [7, 14]. Naturally, this enhances the risk of malaria-related morbidity and fatality. Consequently, parasite drug resistance poses one of the most serious threats to progress in global malaria control and elimination. In human malaria, resistance emerges most frequently against drugs that exclusively target the asexual blood stage [3, 17].

Public Health Consequences

Artemisinin resistance not only delays parasite clearance but also adversely impacts public health [1, 9]. This was first recognized during the emergence of chloroquine and sulfadoxine pyrimethamine resistance, which increased malaria mortality rates by over 20,000 deaths per year and prevented the prevention of 50 million episodes. Despite declining malaria deaths since 2000, resistance may reverse this trend [10]. In Cambodia, where artemisinin resistance first appeared, patients requiring multiple treatment courses often face limited options, intensified time pressure, and a precarious prognosis [14].

Strategies to Combat Resistance

Currently, no single intervention suffices to contain artemisinin resistance because the genetic basis remains elusive [12]. Fluorescence-activated cell sorting facilitates genomic and transcriptomic profiling of resistant parasites; however, these insights have yet to translate into effective drugs, diagnostics, vaccines, or control strategies [14]. Combining artemisinins with partner drugs of distinct mechanisms and extended half-lives in artemisinin-based combination therapies (ACTs) remains the most effective approach to enhance efficacy and stave off resistance [15]. Five WHO-recommended ACT combinations constitute first-line therapy for uncomplicated falciparum malaria: artemether lumefantrine (Coartem), artesunate amodiaquine, artesunate mefloquine, artesunate sulfadoxine-pyrimethamine, and dihydroartemisinin piperaquine. Artemisinins' rapid elimination significantly reduces parasite biomass, enabling partner drugs to clear residual infections and mitigating selection for resistance to either component [14, 15].

Combination Therapies

Combination therapy is the superior strategy for delaying the development of resistance and safeguarding the effectiveness of therapeutic agents [15]. The WHO advocates the use of artemisinin derivatives in conjunction with partner drugs that possess distinct mechanisms of action and longer plasma half-lives. Accordingly, artemisinin-based combination therapies (ACTs) constitute the first-line treatment for uncomplicated falciparum malaria, with five regimens endorsed by the WHO [16]. These combinations pair a rapidly acting artemisinin derivative, which effectuates a swift reduction in parasite biomass during the initial treatment phase, with a longer-acting partner drug responsible for clearing residual parasites and forestalling recrudescence [17]. All key first-line combination therapies presently include an artemisinin derivative. Artemisinin resistance has been documented in Cambodia, Myanmar, Thailand, and Vietnam. The status of P. falciparum susceptibility to artemisinin derivatives is categorized by the WHO into three tiers: Tier I indicates evidence of resistance in clinical isolates; Tier II denotes suspected resistance; and Tier III signifies the absence of any indication of slow parasite clearance. Clinical resistance mechanisms to artemisinin are relatively well-characterized, and a molecular marker associated with the phenotype has been identified [1, 3, 16]. The number of K13 mutant residues correlates positively with the prevalence of resistance at a given site in mainland Southeast Asia. The overall frequency of the K13-propeller mutation is less than 5% in most locations, with outliers reaching up to 47%. Mutations in the kelch 13 gene, therefore, represent the most reliable genome-wide indicator of artemisinin resistance at present [14, 18].

Novel Drug Development

Drug resistance in Plasmodium falciparum poses a major threat to the control and eradication of malaria. The spread of artemisinin-based combination therapies (ACTs) saved millions of lives, yet their efficacy is now threatened by emerging resistance [1, 14]. A robust pipeline of new antimalarials will be necessary, both to prevent the propagation of resistant strains and to ultimately replace current drugs once resistance becomes widespread. To date, the search for replacements has focused mainly on ACT partner drugs, but new compounds

targeting all stages of the parasite's lifecycle are also under development [2, 11]. Plasmodium parasites have a complex lifecycle, transferring between female Anopheles mosquitoes and human hosts. Mosquitoes inject sporozoites into human hosts, which reach the liver and multiply within hepatocytes. Thousands of merozoites are then released into the bloodstream, where they invade red blood cells (RBCs) to begin the blood-stage cycle. Each cycle consists of 48 hours of growth and development culminating in RBC rupture and the release of intracellular merozoites that invade new RBCs [4, 5]. Some parasites commit to sexual development, forming gametocytes that circulate in the bloodstream and complete the lifecycle following uptake by a mosquito. The clinical features of malaria are associated with blood-stage infection, and the main focus of drug development is on compounds capable of killing the blood-stage parasite. One major area of concern is the potential for resistance to spread to other regions. Five markers show emerging resistance to existing antimalarials lumefantrine, amodiaquine, piperaquine, chloroquine, and pyronaridine [8, 9].

Future Directions in Research

Ongoing genome-wide association studies have identified novel candidate loci that could provide insights into artemisinin resistance and design biomarkers for tracking resistant parasite populations at the molecular level [17]. It is crucial to investigate the roles of these genes, as they could reveal unexplored mechanisms of resistance and suggest ways to overcome them [14]. New genomic analysis methods that track the evolution of resistance from early, low-frequency alleles to selective sweeps may aid in the rapid identification of genes involved in drug resistance. The emergence and spread of artemisinin resistance also demands the development of new antimalarial drugs[14, 15]. Several promising candidates appear effective against resistant parasites, including drugs targeting the proteasome, mitochondria, translation, and lysosomes. Importantly, few of these new treatments share mechanisms with artemisinins, so their deployment could limit the emergence of resistance and preserve artemisinin efficacy at the population level [13, 16].

Genomic Studies

Increased efforts to map genetic signatures are introducing novel tools to explore artemisinin resistance. Whole-genome scans associating polymorphisms with parasite clearance data have pinpointed major loci for surveillance [9]. QTL analyses of expression profiles from sensitive and resistant sibling progenies uncover shifts in the transcriptional architecture of gene regulation: clusters of cis- and trans-acting loci modulate sensitivity to antimalarial drugs. Comparative genome hybridization reveals copy number variations linked to elevated clearance half-lives [10]. At least three distinct resistance mechanisms shape the adaptive landscape, each exerting unique selective pressures and generating genomic profiles that assist field monitoring of sensitive and resistant populations. Resistance traits are also inherited through hybrid and backcross crosses, with forward genetic mapping identifying genomic loci that contribute to drug response [2, 8]. Deployment of selective compounds has facilitated the large-scale selection of recombinant progeny pools, enabling bulk segregant analysis of pooled phenotypes and rapid identification of resistance determinants [13, 18]. The genetic architecture of resistance is elucidated by multiple experimental and analytical strategies that closely align with patterns emerging from field studies [3, 4, 5]. In vitro-derived resistant parasites lacking mutations in known candidates reveal a novel molecular marker and resistance-conferring locus emerging independently of the current PfKelch mechanism [12, 14]. A chemical pulsing approach replicates ex vivo clearance dynamics and extends the timeline to examine the onset of stalls and recrudescence [2]. Genomic analysis identifies the sec14 domain-containing phospholipid transporter (pfcarl) as the major locus associated with resistance at later stages. Understanding the genetic architecture of artemisinin resistance informs the development of high-resolution tools essential for genomic surveillance [1, 11].

Innovative Therapeutic Approaches

Several interventions are under investigation to overcome resistance. Strategies include combining artemisinins with novel partner drugs or agents that block resistance mechanisms. Repurposing existing drugs also offers an alternative route [17]. Genome editing has the potential to explore drug targets and susceptibilities, thereby guiding the design of new therapies [5].

Policy Implications

The emergence of drug resistance to artemisinins poses a new challenge for global malaria control. Since the discovery of artemisinin resistance, the global public health and research communities have mobilized efforts in the form of new policy, new funding, and new initiatives to preserve the effectiveness of artemisinin-based therapies and to limit the spread of resistance [14]. The importance of fostering research to increase the understanding of resistance mechanisms remains a priority [12]. A global research network termed ARTEMIP, which aims to exploit multidisciplinary expertise and state-of-the-art technologies to describe the resistance mechanism and to

devise countermeasures, has been assembled [17]. Additional resources have been assembled to provide a global infrastructure to support these activities.

Global Health Initiatives

The utilization of antimalarial medicines in both prophylaxis and treatment constitutes an essential public health framework of malaria reduction and elimination programs [2, 16]. Policy guidance provided by the World Health Organization (WHO) supports appropriate spatial and temporal strategies, selection of optimal chemical regimens, and community engagement [1, 17]. Further, a key role of funding opportunities such as those awarded by the Global Fund to Fight AIDS, Tuberculosis and Malaria, or the President's Malaria Initiative is to provide financial mechanisms that support national strategies and meet procurement needs, thereby stabilizing distribution chains and improving access to medicines [1, 16].

Funding and Resource Allocation

The discovery of antimalarial drugs is a lengthy process that involves many more years of laboratory and clinical development and can cost billions of dollars [12, 18]. The importance of preserving the efficacy of current regimens and artemisinin derivatives is therefore fundamental to malaria control and eradication efforts [14]. Thus, artemisinin resistance should be regarded as a global challenge that demands political involvement and specific funding strategies to support research and development and to help malaria-endemic countries control the spread of resistance [14, 18].

CONCLUSION

Artemisinin resistance is a multifactorial phenomenon driven by genetic mutations, biochemical adaptations, and cellular stress responses in Plasmodium falciparum. Its spread threatens malaria elimination efforts globally, particularly in Africa, where the disease burden is highest. Safeguarding the efficacy of ACTs requires an urgent and coordinated response, including robust surveillance systems, the development of next-generation antimalarials, and integrated public health strategies. Continued investment in research and global collaboration is critical to prevent the rollback of decades of malaria control progress.

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