Polyclonal Malaria Infections and Drug Resistance Evolution: Implications for Treatment Strategies

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Abstract
Malaria remains one of the most significant public health challenges globally, with sub-Saharan Africa and particularly Nigeria, bearing a disproportionate share of the disease burden. Despite advances in vector control and the adoption of artemisinin-based combination therapies (ACTs), the rapid emergence and spread of drug-resistant Plasmodium falciparum strains threaten recent gains in malaria control. A critical yet underexplored factor accelerating this resistance evolution is the presence of polyclonal infections, wherein multiple genetically distinct parasite strains coexist within a single host. These mixed-strain infections foster within-host competition, facilitate recombination events in the mosquito vector, and increase the probability of survival for resistant clones under drug pressure. This review synthesizes current knowledge on the biological and epidemiological underpinnings of polyclonality, its role in resistance dynamics, and its unique significance in the Nigerian context, where high transmission intensity and suboptimal treatment practices amplify the problem. We highlight diagnostic limitations in detecting minority resistant clones, the clinical consequences of recrudescence and therapeutic failure, and the role of polyclonal carriers as potential super spreaders of resistant parasites. Furthermore, we examine current resistance trends in Nigeria, discuss surveillance and treatment gaps, and propose integrated strategies encompassing molecular monitoring, diagnostic improvements, optimized treatment policies including triple ACTs and vector control measures. By identifying knowledge gaps and synthesizing evidence across clinical, epidemiological, and molecular dimensions, this review underscores the urgent need for multifaceted interventions tailored to Nigeria's malaria ecosystem. Addressing polyclonal infections in resistance management is critical to sustaining the efficacy of frontline therapies, protecting vulnerable populations, and advancing global malaria elimination goals.

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1. Introduction

Malaria still poses a global public health threat. The rate of morbidity and mortality from malaria has risen in several parts of the world, as reported in the World Malaria Reports, which documented over 200 million confirmed cases and more than 400, 000 deaths in 2019 [1-3]. The spread of Plasmodium falciparum resistance to chloroquine and sulfadoxine–pyrimethamine has

been revealed through molecular epidemiological studies. Parasite strains with high levels of resistance to these drugs originated in Asia and later reached Africa, causing severe impacts on human health and survival [4].

The cycle of Plasmodium transmission between human and mosquito hosts, and its ability to produce gametocytes, is crucial. When a mosquito ingests gametocytes during a blood meal from an infected human, they develop into gametes that undergo sexual recombination to form zygotes or ookinetes, which then cross the mosquito's midgut wall and mature into oocysts. This process can be influenced by factors namely host immunological responses, gametocyte viability and density, and the complexity of the infection [5-9].

Due to the growing spread of insecticide and antimalarial drug resistance, efforts to eliminate malaria have faced major challenges. These difficulties are further compounded by the complex nature of parasite populations and the absence of a widely effective vaccine [2, 10-15].

People are commonly infected with numerous P. falciparum parasite strains at the same time in areas where infections are endemic, a condition known as polyclonal infections. These can occur as a result of serial superinfections, in which one or more clones are spread by successive mosquito bites, or clonal co-transmission, in which a single insect introduces numerous clones. Furthermore, meiotic recombination of P. falciparum parasites within Anopheles mosquitoes contributes to the emergence of novel alleles and the generation of new strains, a cycle that is likely to persist as long as vectors, parasites, and human hosts coexist [16-18].

Understanding Plasmodium epidemiological patterns and transmission dynamics requires knowledge of the multiplicity of infection (MOI) and/or the complexity of infection (COI). Infections typically show higher multiclonality in areas where malaria transmission is high, particularly among asymptomatic individuals. The different parasite clones compete with one another for replication and transmission, though the resources they compete for are not clearly understood [19-20].

Understanding how polyclonality influences the evolution of drug resistance is fundamental to shaping effective malaria treatments. This knowledge not only supports better drug policy and combination therapy decisions but also strengthens surveillance systems and informs vaccine development. However, it remains unclear whether multiclonal infections arise because natural immunity is weakened—allowing multiple genetically distinct clones to thrive—or because new parasite populations are introduced into communities. Knowing how these infections interact with host immune responses is therefore vital for predicting clinical malaria risks and evaluating the success of control measures. In this review, we examine the role of polyclonal malaria infections in driving resistance patterns and consider how these insights can guide more resilient and sustainable strategies for malaria treatment and control.

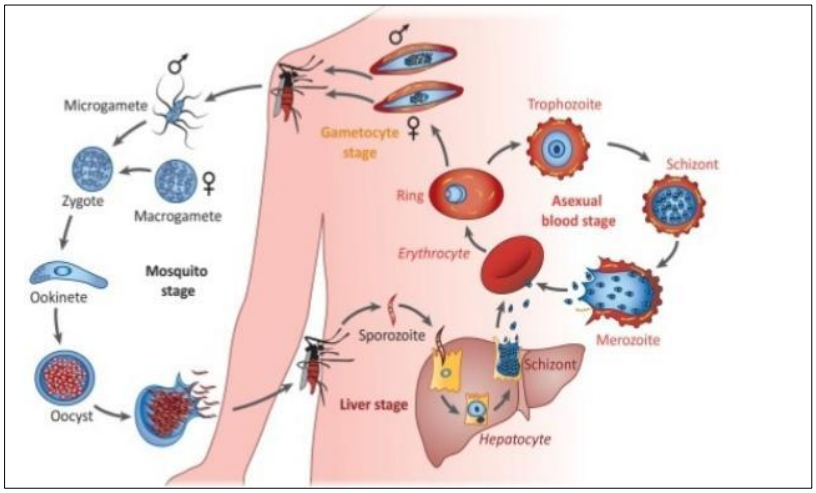

Fig 1: The life cycle of Plasmodium falciparum [21]

2. Polyclonal Malaria Infections: Biological Basis and Epidemiology

2.1. Definition and Characteristics

Polyclonal malaria infections occur when an individual harbors multiple genetically distinct clones of the same Plasmodium species. They may originate through co-transmission, when a mosquito inoculates different clones in a single bite, or through repeated exposure to infected mosquitoes (superinfections) [22].

Novel alleles in P. falciparum arise from meiotic recombination within Anopheles mosquitoes, generating new strains. This cycle continues as long as parasite, human host, and vector populations coexist. The evolution of drug-resistant genes has further accelerated the genetic diversity of malaria parasites. Patients infected with multiple P. falciparum strains are more likely to experience treatment failure compared to those infected with fewer strains [23].

Molecular detection of polyclonality relies on two main approaches, depending on the markers used [24, 25]:

1. Traditional genotyping of highly polymorphic loci (msp-1, msp-2, glurp, microsatellites), which distinguishes alleles by fragment size.
2. Amplicon deep sequencing and SNP barcoding, which detect minority clones at frequencies as low as 0.5%.

Polyclonal malaria infections exhibit several distinct characteristics:

1. **High Multiplicity of Infection (MOI):** The key feature of polyclonal infections. MOI refers to the number of distinct parasite clones in a single infection, while complexity of infection (COI) describes their genetic traits. Polyclonal infections are also termed "multiple clone infections" [26].
2. **Host and Immune Response Factors:** Polyclonality

increases with parasite density but declines with age due to acquired immunity. In Gabonese children, higher MOI was linked to weaker antibody responses and greater clinical risk, while in adults, immunity reduced disease severity despite multiple clones [22, 27, 28].

3. **Transmission and Evolutionary Implications:** Minority clones can carry drug-resistance alleles and be transmitted by mosquitoes, even at low densities. In Mali, resistant clones sometimes gained a transmission advantage even after ACT treatment (Malaria Journal, 2025) [29]. Recombination within mosquitoes further enhances diversity and complicates vaccine development due to antigenic variation [30].
4. **Prevalence and Diversity:** These infections are common, ranging from 0–99% in P. falciparum and nearly 100% in P. vivax. MOI varies with geography and transmission intensity, from 1 to over 6 (Lopez & Koepfli, 2021) [26]. In Western Kenya and 1.7–1.9 in Tanzania. In Northwest Ethiopia, MOI strongly correlated with parasite density but not host age [31, 32].

2.2. Drivers of Polyclonality

Mosquito transmission dynamics are a major driver of polyclonality. Polyclonal infections are mostly prevalent in endemic regions and are highly conducive to drug resistance, as their occurrence is shaped by biological, epidemiological, and environmental factors [26].

Geographic variation adds another layer. In North Central Nigeria, 88% of P. falciparum infections were polyclonal, reflecting high allele diversity [23]. By contrast, in Northwest Ethiopia, multiplicity of infection correlated more with parasite density than with host age [32].

Parasite density also influences detection. High parasite loads make minority clones more visible, while low-density infections may appear monoclonal due to diagnostic limitations [32]. Epidemiological and ecological factors create the conditions for polyclonality, and understanding molecular mechanisms is fundamental in explaining how resistance undermines therapy.

2.3. Nigerian Epidemiological Context

In Nigeria, there is a prevalence of Plasmodium falciparum. In North Central Nigeria, genetic diversity and malaria prevalence may have risen due to large-scale migration between 2009 and 2013 following the insurgency in Northeastern Nigeria. Research carried out in this region on 282 samples collected showed the presence of 39 msp1, 31 msp2, and 13 glurp alleles, with 88% of infections being polyclonal. A significant proportion of samples contained trimorphic and dimorphic allele combinations, indicating high infection complexity. These results demonstrate how high genetic diversity accelerates the evolution of drug-resistant genes. To prevent the spread of P. falciparum and reduce the risk of drug resistance emerging in Nigeria, stronger measures are required, including improved drug quality and diagnostics [23].

Similarly, in the Ota area of Southwestern Nigeria, studies using blood and corresponding saliva samples collected from 1, 243 febrile subjects of all ages and sexes revealed Pfcrt in 57.42% of blood samples and 51.02% of saliva samples. Microscopically confirmed cases showed that 34.78% of individuals carried the Pfmdr^{-1} mutated gene, while 26.67% of saliva samples also revealed its presence. Epidemiological analysis further showed wild-type Pfk13 genes in 21.84% of blood samples and 44.44% of saliva samples [33].

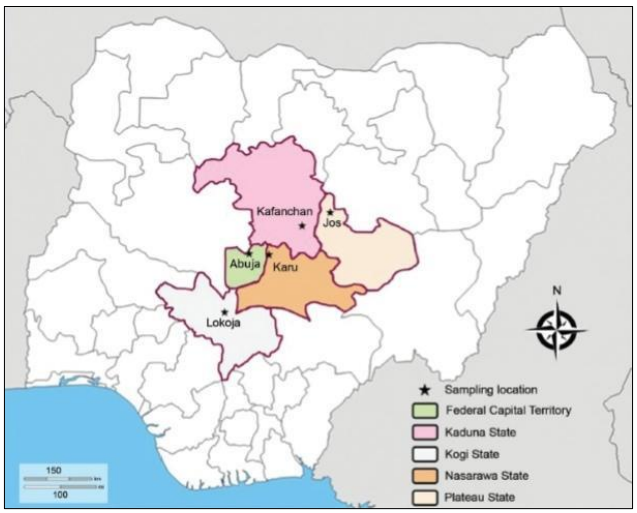

Fig 2: The epidemiology of Plasmodium falciparum in North Central Region of Nigeria [23]

3. Molecular Basis of Antimalarial Drug Resistance

3.1. Key Resistance Markers

Molecular markers for parasite susceptibility to antimalarial drugs include:

1. Plasmodium falciparum multidrug resistance 1 (PfMDR1)
2. Plasmodium falciparum Ca^{2+}-ATPase (PfATP6)
3. Plasmodium falciparum Chloroquine resistance transporter (PfCRT)
4. Plasmodium falciparum Kelch^{-1}3 propeller domain (PfK13) loci
5. Pfdhfr

Mutations in these molecular markers influence antimalarial drug resistance. For instance, mutation in PfCRT is responsible for chloroquine, amodiaquine, and piperaquine resistance. This transporter, located on the membrane of the digestive vacuole, in its variant form's exports weak-base $^{4-}$ aminoquinoline drugs from the acidic organelle, preventing their binding to heme and thereby blocking heme detoxification [34].

The emergence of artemisinin-resistant Plasmodium falciparum strains in Southeast Asia raises major concerns about their potential spread to Africa. According to current World Health Organization (WHO) guidelines, artemisinin-based combination therapies (ACTs) remain the recommended first-line treatment for severe malaria [35].

Artemisinin resistance is associated with mutations in several parasite genes. The PfMDR1 locus on chromosome 5, through copy number variations, contributes to multidrug resistance, including reduced sensitivity to artemisinin. The PfATP6 gene on chromosome 1, which encodes a calcium pump once suggested as a potential drug target, has also been investigated as a marker of artemisinin resistance. More recently, mutations in the PfK13 propeller region on chromosome 13 have been strongly linked to clinical resistance in Cambodia and to *in vitro* ring-stage survival. These variants have since spread widely across Southeast Asia and South China and have also arisen independently in other regions. As a result, alterations in PfATP^{6,} PfMDR^{1,} and PfK13 are regarded as key molecular indicators of artemisinin resistance [36-39].

3.2. Mechanism of Selection

In Africa, normal treatment for falciparum malaria changed to artemisinin-based combination therapy (ACT) early this century because earlier drugs became less effective [40]. ACT combines a potent fast-acting artemisinin derivative with a slower-acting partner drug. After artemisinins act on most of the parasites, the partner drugs are essential for eradicating remaining parasites and help prevent the establishment of resistance. ACT therapy includes artemether-lumefantrine, artesunate-amodiaquine, dihydroartemisinin-piperaquine, artesunate-mefloquine, and artesunate-pyronaridine. However, Southeast Asian artemisinin resistance [41] and partner drug variability pose a danger to ACT effectiveness [42].

Partner drugs often display opposing sensitivity profiles due to polymorphisms in putative drug transporters [41]. For instance, the PfCRT 76T mutation confers resistance to both amodiaquine and chloroquine [42, 43], while the K76 wild type is linked to decreased sensitivity to lumefantrine and mefloquine [43]. Similarly, PfMDR1 N86Y exhibits a comparable pattern: 86Y is selected by amodiaquine therapy and linked to decreased sensitivity to amodiaquine and chloroquine, while N86 is selected by lumefantrine therapy [46–49] and linked to decreased sensitivity to lumefantrine and mefloquine [43, 14].

Shifts in transporter mutations under drug pressure reveal how drug sensitivity and parasite fitness interact to shape circulating genotypes. This dynamic is particularly pronounced in polyclonal malaria infections, where treatment removes drug-sensitive parasites but allows resistant ones to persist, multiply, and dominate. In this way, polyclonality creates the environment for within-host competition that fuels the mechanism of selection.

The fitness effects of PfMDR1 haplotypes remain uncertain. Co-culture studies found wild type parasites outgrowing mutants like 184F, 1034C, 1042D, and 1246Y, but not 86Y, which seems particularly important in Africa [44]. In the Gambia, N86 wild type dominated early in the season under low drug pressure [45], whereas in Uganda, 86Y alleles expanded faster, suggesting a fitness edge possibly through interactions with other PfMDR1 mutations [29, 30]. Isogenic haplotype experiments further confirmed that these mutations directly shape parasite fitness.

4. Drug Resistance Trends

David Clyde conducted fieldwork in Tanzania in the 1950s, where pyrimethamine was given once a month to inhabitants of a rural village for the ability of the drug to cure Plasmodium falciparum infection. There were no treatment failures at first, but by the third month, 8% of infections were still present, and by the fifth month, that number had increased to 37%. Within a year, weekly dosing resulted in resistance rates of 50% or above [50]. It was eventually determined that point mutations in P. falciparum dihydrofolate reductase (DHFR) were the source of the localized and contiguous spread of resistance [51].

Sulfadoxine-pyrimethamine failures were explained by the progressive accumulation of DHFR and DHPS mutations [52, 53].

In contrast, resistance to chloroquine only appeared twice in the late 1950s and gradually spread from the Thai-Cambodian and Panama-Colombian borders [54]. By the 1970s, it had spread to India, and by 1978, to Africa. Its efficacy in East Africa was compromised by the 1990s, while it lasted longer in West Africa. K76T was central among the several pfcrt mutations that induced resistance. K76T was crucial to the resistance caused by multiple pfcrt mutations [55, 56].

Subsequent investigations in Mali demonstrated the swift selection of DHFR mutations under sustained drug pressure [57]. Further genetic studies showed that highly resistant DHFR and DHPS variants originated from only a few ancestral lineages, which then disseminated globally in a pattern resembling chloroquine resistance [58, 59]. In Malawi, chloroquine resistance declined and vanished within eight years of its discontinuation, driven by the re-expansion of wild-type pfcrt parasites, highlighting the fitness disadvantage associated with resistant strains in the absence of drug pressure [60, 61].

These lessons emphasize that in order to stop resistance from spreading globally, it must be identified early. Concerns have been raised by reports of declining ACT efficacy in Southeast Asia, even though documented artemisinin resistance has not yet been established [62]. Molecular processes and surveillance markers are being identified through genomic techniques [63]. Should artemisinin resistance emerge, swift containment at its source—such as mass screening and treatment in western Cambodia—will be vital to protect ACT effectiveness and avert a potentially devastating global spread.

4.1. Contributing Factors

Parasite biology is not the only epidemiological domain that offer insights and explanation for the persistence of antimalarial drug resistance across Nigeria; systemic and behavioral variables are also important factors that contribute to this. The widespread distribution of counterfeit and inefficient antimalarial medications, which compromise therapeutic efficacy and promote partial parasite clearance, is one of the main causes. In addition to this, poor-quality artemisinin-based combination treatments (ACTs) are widely available in West Africa, as highlighted by several epidemiological studies, with several estimates ranging from 10 to 30 percent of sampled medications that fail phytochemical analysis or package authenticity checks [64, 65]. Products like this give resistant clones an ecological niche to thrive in, in addition to exposing parasites to sub-therapeutic medication doses.

Another important aspect is the prevalence of informal drug use and self-medication in Nigeria, which is strongly attributed to a defective health system infrastructure, difficult access to trained healthcare professionals, and expensive out-of-pocket expenses for medication costs. Many patients buy incomplete dosages or treatment packages from patent pharmaceutical vendors (PMVs) or start treatment with leftover medications [66, 67]. Due to the uneven drug exposure caused by these treatment patterns, resistant parasites are more likely to prevail in polyclonal infections. Furthermore, this challenge is worsened by cultural inclinations for quick symptom relief over complete therapy adherence [68].

Although self-medication and incomplete treatment courses are intimately related, poor patient adherence occurs even when ACTs are provided appropriately. According to studies, between 25 and 40 percent of patients in Nigeria do not complete the entire 3-day ACT regimen [69, 70]. This leads to the reappearance of Minority resistant strains, a situation directly linked to incomplete clearance, especially in situations with high multiplicity of infection (MOI), where

drug exposure can alter inter-strain competition in favor of resistant parasites.

Lastly, one institutional factor contributing to the persistence of resistance and parasite evolution is the lack of efficient prescribing procedures and practices of healthcare professionals. Monotherapies, including artemisinin derivatives or residual chloroquine stocks, are sometimes prescribed in both public and private facilities, even though they are prohibited by Nigerian healthcare policy [71, 72]. In addition to impairing patient outcomes, inappropriate combinations, underdosing, and departures from national treatment standards facilitate the emergence of resistance genotypes. Most importantly, these behaviors persist due to non-indulgence of drug enforcement and regulation agencies as well as poor pharmacovigilance [73].

5. Clinical and Public Health Implications

Polyclonal malaria infections have direct effects on the handling and management of clinical cases, surveillance systems, and more general public health initiatives, with ramifications that go well beyond the laboratory. Polyclonality in Nigeria and West Africa at large adds levels of complication to malaria control in places, where high endemicity, poor surveillance, and extensive self-medication already pose a serious challenge and difficulty. These difficulties fall into three primary categories: transmission dynamics, treatment results, and diagnostic limits.

5.1. Diagnostic Challenges

A precise diagnosis of malaria is essential for both efficient therapy and monitoring. Nevertheless, polyclonal infections compromise not only the sensitivity of the diagnostic methods but also the specificity of the procedures in use, including microscopy and rapid diagnostic tests (RDTs).

Rather than identifying different parasite lineages, RDTs are intended to identify the existence of an infection by detecting antigens such as parasite lactate dehydrogenase (pLDH) or histidine-rich protein 2 (HRP2) [74]. Submicroscopic concentrations of parasite subgroups that are resistant may be present in the case of polyclonal infections. Despite an overall positive test result, resistant parasites are often underreported as these minority clones are often not captured by RDTs [75]. Furthermore, RDT reliability is additionally complicated by the rising incidence of *pfhrp2* and *pfhrp3* gene deletions. Cases like this are predominant in Nigeria and several African nations with high prevalence of malaria, a situation that potentially lead to the alteration of results such as the production of false negatives regardless of parasite density [76].

Similar limitations apply to light microscopy, which holds wide adoption as a standard reference model in regions of high endemicity. Although they are unable to resolve genetic variation within infections, microscopists can estimate parasitemia. Therefore, this often results in a longer detection time or identification window of growing drug resistance. This is especially possible if the resistant subpopulations, even though sensitive, remain undetected [77].

A now widely adopted technique for detecting low-frequency resistant clones involves the use of molecular techniques such whole-genome sequencing and amplicon deep sequencing. In many other cases, PCR genotyping has also been found to be very effective [78, 79]. Due to high cost of equipment procurement, and other factors which include lack of qualified staff, these methods are still mostly unavailable in Nigeria [80]. As a result, polyclonality's diagnostic blind spots impair both national surveillance and patient-level care.

Table 1: Diagonistic methodologies for polyclonal malaria

Method/Procedure	Limit of detection (LOD)	Potency in detecting minority resistant clone	Strengths	Shortfalls & Feasibility in Nigeria
HRP^{2-}based RDT	~70–100 parasites/µL	Weak. minority clones below LOD and HRP^{2-}deleted parasites undetectable	Affordable	Cases of false negatives are prevalent with HRP2 deletions; obtainable in primary care
Light Microscopy	~100–500 parasites/µL (expert may detect ~10/µL)	Poor; Only captures dominant clones	Species identification is possible	Quality varies across facilities
Whole genome sequencing (WGS)	low sensitivity (especially at low density) unless deep coverage	Effective for comprehensive resistance mapping	Perfectly applicable to population genomics	Extremely expensive
ddPCR	to a few copies per µL; superior quantitation	Excellent sensitivity for low-frequency targets	Excellent for copy-number and HRP2 deletion assays	Costly equipment and set-up; still niche application
Amplicon deep sequencing	Suitable for detecting minority alleles; can also genotype low density samples	Excellent; reconstructs haplotypes and minority variants	Perfect for recrudescence vs reinfection	Works best as reference; requires sequencing

5.2. Treatment Outcomes

Polyclonality has crucial therapeutic implications. The most effective treatment for sensitive strains in Nigeria at the moment is artemisinin-based combination treatments (ACTs). Nonetheless, upon medication exposure, resistant bacteria might stay resistant within-host competition, an incidence that is linked to polyclonality [81]. Sensitive strains are eliminated on initial exposure to treatment; however, treatment failure may result from resistant minority populations that then begin to grow and proliferate, eventually spreading their colony across the host's blood tissue.

Clinically, this occurs as recrudescence, which is defined as the recurrence of parasitemia following a time of initial clearance [82]. It can be challenging to experts to differentiate between recrudescence and reinfection, which is a condition where new parasites are acquired following treatment, particularly in high-transmission environments like Nigeria. This distinction is often established by genotyping polymorphic markers like *glurp*, *msp-1*, and *msp-2* [83].

Nonetheless, in regions where high multiplicity of infection (MOI) is recorded or possible, factors such as overlapping allelic profiles overpower the screening efficacy of these markers, which then leads to misclassification [84]. This uncertainty in detection directly affects the investigations of therapeutic efficacy. The perceived efficacy of ACTs can be inflated by misclassifying recrudescence as reinfection, which could impact policy adjustments and increase the likelihood of proliferation of resistant strains [85]. In addition, recrudescence increases the risk of anemia, lengthens morbidity, and places additional financial and medical strain on patients. Maternal anemia, stillbirth, and low birthweight are linked to treatment failure in pregnant women in regions of high endemicity [86].

Furthermore, the survival and spread of resistant clones are enhanced by recurrent sub-curative exposure to ACTs, which can result from inadequate adherence, substandard medication and treatment, or improper prescription [87]. These circumstances hasten the selection of resistance alleles in polyclonal environments and erode the effectiveness of ACT.

5.3. Transmission Dynamics

From a public health point of view, the transmission effects of polyclonality might be the most worrisome. People who have polyclonal infections may disseminate resistant and sensitive strains throughout the community by acting as "superspreaders" of resistance. In the midgut of mosquitoes, genetic recombination takes place when gametocytes from several clones are ingested [88]. Resistant haplotypes that are novel and that may contain combinations of resistance alleles absent from the original infection are made possible by this recombination [89]. This process greatly speeds up the spread of resistance within the parasite population in high-transmission environments.

Another concerning factor in malaria prevalence is the presence of asymptomaticity in Nigeria, which is an important consideration often overlooked by experts, stakeholders and public health specialists. Multiple parasite clones, including resistant ones, are commonly present in subclinical infection patients, and are known to spread silently [90]. Hence, asymptomatic carriers serve as reservoirs for the retainment and dissemination of resistance at the community level, a situation attributed to rarity of examination and treatement [91].

Vector control measures are additionally compromised by the existence of minority clones that are effectively resistant in polyclonal infections. Future outbreaks may be sparked by persisting resistant subpopulations even if LLINs and IRS lessen the intensity of transmission, particularly when medication policy or vector interventions are not radically implemented [92].

6. Strategies and Future Directions

A thorough, long-term approach is required in Nigeria and West Africa due to the persistence of polyclonal malaria infections and their contribution to the acceleration of drug resistance. In order to ensure active community engagement, future treatments must incorporate molecular surveillance, improved vector control, innovative diagnostics, and optimized treatment policies.

6.1. Surveillance and Monitoring

To detect new resistance alleles and track their distribution, molecular surveillance is essential. Actionable insights into parasite evolution are offered by resistance markers such *pfcrt*, *pfmdr1*, *pfdhfr*, *pfdhps*, and *pfkelch13* [93]. Early warning systems that can direct prompt policy changes before widespread treatment failure occurs are made possible by integrating molecular surveillance into programs aimed at national control of malaria [94].

Additionally, incorporating multiplicity of infection (MOI) data into standard epidemiological evaluations is undeniably crucial. Two factors –the risk of polyclonal infections and transmission intensity are both reflected in MOI, which is measured using genotyping markers such as $msp^{-1,}$ $msp^{-2,}$ and glurp [95]. Tracking MOI provides significant data on how transmission patterns contribute to resistance spread, allowing policymakers a fuller understanding of circumstances relating to the infection and its epidemiology [96]. In order to give a cohesive picture of resistance throughout West Africa, strengthening surveillance necessitates funding laboratory facilities, developing local scientists' capacity, and encouraging regional data-sharing networks [97].

6.2. Diagnostic Improvements

Recurrent studies done locally have shown that the intricacy of polyclonal infections cannot be resolved by current diagnostic techniques. Although dependable RDTs and quality-assured microscopy must continue to be the cornerstones of malaria diagnosis, it is impossible to ignore their shortcomings in identifying minority resistant clones [98]. To reduce low-quality testing equipment and environments, it is essential to expand investment in quality control methods, which include standardizing RDT procurement and providing microscopists with frequent refresher training [99].

Molecular assays such as loop-mediated isothermal amplification (LAMP) and PCR if adopted at local levels have enormous prospects. However, they are prone to suffer setbacks as implementation often demands affordability, simplification and also prospects to scale in resource-limited settings [100]. New technologies like CRISPR-based diagnostics and nanopore sequencing hold potential for field-deployable, highly sensitive detection tools [101]. If adequately adapted, these innovations could bridge the gap between advanced laboratories and frontline clinical settings in Nigeria. Future solutions lie in the development of next-generation diagnostics that can detect mixed infections at submicroscopic levels.

6.3. Optimizing Treatment Policies

Strict adherence and strong dedication to dosage regimen are necessary to guarantee ACT efficacy. The first priority should be to enforce ACT conformity. Incomplete treatment routines and procedures that fuel resistance can be lessened with the help of follow-up systems, mobile health reminders, and community health education [102].

The second requirement is to completely eradicate monotherapies. Because of lax regulation and over-the-counter sales drugs like chloroquine, artesunate, and sulfadoxine-pyrimethamine monotherapies remain in circulation in some parts of Nigeria in defiance of WHO guidelines [103]. To stop monotherapy-driven resistance, stricter regulations on pharmaceutical companies and sanctions against unlicensed medication sellers are necessary.

Third, new treatment strategies that are ripe for exploration are triple ACTs (TACTs). This treatment regimen combines two partner medications with artemisinin derivatives, present a viable way to tackle the formation of resistance. Combinations of artemisinin, piperaquine, and mefloquine as well as artemether, lumefantrine, and amodiaquine have demonstrated improved efficacy and durability in trials [104, 105]. A vital safety measure for the treatment of malaria might be offered by incorporating TACTs into public health policies for high-resistance areas in Nigeria.

6.4. Integrated Malaria Control

Long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), and larval source management are vector control strategies that continue to be the cornerstones of malaria prevention. [106], However, factors such as insecticide resistance and inconsistency in use limits feasibilty of containment efforts [107]. Innovations like dual-active ingredient LLINs and rotational spraying with multiple insecticide classes are required to sustain effectiveness [108]. Addressing drug resistance in polyclonal settings necessitates an integrated control approach that goes beyond pharmaceuticals.

Involving the community is equally important. Campaigns for public awareness should emphasize adherence to recommended regimens, discourage self-medication, and emphasize reasonable drug usage [109]. Ownership and sustainability are promoted by local involvement in vector control measures such as stringent environmental management and reduction of larval source [110]. The best chance to reduce the emergence of resistance and maintain the effectiveness of frontline therapies is to take an integrated approach that links robust vector management, active surveillance, powerful diagnostics, and effective treatment.

7. Conclusion

Especially in endemic areas like Nigeria, polyclonal Plasmodium falciparum infections are a significant but frequently overlooked cause of antimalarial medication resistance. Multiple parasite clones living together in the same host promotes genetic diversity, raises the possibility of resistant alleles developing, and makes treatment responses more challenging. [111] Strides made in the control and eradication of malaria may be undermined in such environments, where resistant strains may continue to exist despite combination treatments.

Polyclonal infections have clinical repercussions that transcend beyond the failure of specific treatments. Due to diagnostic constraints, resistant minority clones are often missed, which results in incorrect treatment outcome classification and an underestimation of the prevalence of resistance. Whether from reinfection or recrudescence, recurrent infections complicate clinical judgment and impede surveillance efforts. Polyclonal carriers act as transmission amplifiers at the population level, hastening the spread of resistant groups both within and between communities. [112, 113]

A multifaceted approach is needed to address this challenge: Increasing diagnostic accuracy, integrating multiplicity of infection (MOI) metrics into epidemiological monitoring, and strengthening molecular surveillance of resistance markers will be essential for timely detection of emerging resistance. Also, on the therapeutic front, strict adherence to artemisinin-based combination therapies (ACTs) should see remarkable progress on containment [114, 115]. Policy making also requires enforcement of drug quality standards, and investigation of novel regimens like triple ACTs (TACTs) that provide promising avenues on improvement of drug composition. On the other hand, community-driven education campaigns that discourage self-medication practices and encourage rational drug use through the use of long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS), and environmental management.

With Nigeria accounting for some of the highest prevalence in Africa, presents both the greatest risk and the greatest opportunity for intervention. By investing in strong surveillance systems, improving diagnostic and therapeutic pathways, and integrating community participation in malaria control, stakeholders can reduce the risks posed by polyclonal infections. In the end, the interaction between polyclonality and resistance evolution highlights the need for a stronger integration of clinical, molecular, and public health strategies. If global elimination targets are to remain within reach, collaboration among researchers, clinicians, policymakers, and communities will be essential.

8. References

1. World Health Organization. World malaria report 2019. Geneva: World Health Organization; 2019.
2. World Health Organization. World malaria report 2020: 20 years of global progress and challenges. Geneva: World Health Organization; 2020.
3. World Health Organization. World malaria report 2023. Geneva: World Health Organization; 2023.
4. Christopher VP. The evolution of drug-resistant malaria. Trans R Soc Trop Med Hyg. 2009;103(Suppl 1):S11-4. doi:10.1016/j.trstmh.2008.11.002
5. Morlais I, Nsango SE, Toussile W, Abate L, Annan Z, Tchioffo MT, *et al*. Plasmodium falciparum mating patterns and mosquito infectivity of natural isolates of gametocytes. PLoS One. 2015;10(4):e0123777. doi:10.1371/journal.pone.0123777
6. Nsango SE, Abate L, Thoma M, Pompon J, Fraiture M, Rademacher A, *et al*. Genetic clonality of Plasmodium falciparum affects the outcome of infection in Anopheles gambiae. Int J Parasitol. 2012;42(6):589-95. doi:10.1016/j.ijpara.2012.03.008
7. Molina-Cruz A, Canepa GE, Alves e Silva TL, Williams AE, Nagyal S, Yenkoidiok-Douti L, *et al*. Plasmodium falciparum evades immunity of anopheline mosquitoes by interacting with a Pfs47 midgut receptor. Proc Natl Acad Sci U S A. 2020;117(5):2597-605. doi:10.1073/pnas.1917042117
8. de Jong RM, Tebeje SK, Meerstein-Kessel L, Tadesse FG, Jore MM, Stone W, *et al*. Immunity against sexual stage Plasmodium falciparum and Plasmodium vivax parasites. Immunol Rev. 2020;293(1):190-215. doi:10.1111/imr.12828
9. Stone WJR, Campo JJ, Ouedraogo AL, Meerstein-Kessel L, Morlais I, Da D, *et al*. Unravelling the immune signature of Plasmodium falciparum transmission-reducing immunity. Nat Commun. 2018;9:558. doi:10.1038/s41467-018-03045-2
10. Dhorda M, Amaratunga C, Dondorp AM. Artemisinin and multidrug-resistant Plasmodium falciparum - a threat for malaria control and elimination. Curr Opin Infect Dis. 2021;34(5):432-9. doi:10.1097/QCO.0000000000000766

11. Huang W, Cha S, Jacobs-Lorena M. New weapons to fight malaria transmission: a historical view. Entomol Res. 2022;52(6):235-40. doi:10.1111/1748-5967.12580
12. Maharaj R, Kissoon S, Lakan V, Kheswa N. Rolling back malaria in Africa - challenges and opportunities to winning the elimination battle. S Afr Med J. 2019;109(11b):53-6. doi:10.7196/SAMJ.2019.v109i11b.14246
13. Buckee CO, Gupta S. Modelling malaria population structure and its implications for control. In: Michael E, Spear RC, editors. Modelling parasite transmission and control. New York: Springer; 2010. p. 112-26. (Advances in Experimental Medicine and Biology; vol. 673).
14. Naung MT, Martin E, Munro J, Mehra S, Guy AJ, Laman M, *et al*. Global diversity and balancing selection of 23 leading Plasmodium falciparum candidate vaccine antigens. PLoS Comput Biol. 2022;18(2):e1009801. doi:10.1371/journal.pcbi.1009801
15. Ouattara A, Barry AE, Dutta S, Remarque EJ, Beeson JG, Plowe CV. Designing malaria vaccines to circumvent antigen variability. Vaccine. 2015;33(52):7506-12. doi:10.1016/j.vaccine.2015.09.110
16. Conway DJ, Roper C, Oduola AMJ, Arnot DE, Kremsner PG, Grobusch MP, *et al*. High recombination rate in natural populations of Plasmodium falciparum. Proc Natl Acad Sci U S A. 1999;96(8):4506-11. doi:10.1073/pnas.96.8.4506
17. Meyer CG, May J, Arez AP, Gil JP, do Rosario V. Genetic diversity of Plasmodium falciparum: asexual stages. Trop Med Int Health. 2002;7(5):395-408. doi:10.1046/j.1365-3156.2002.00875.x
18. Kiwanuka GN. Genetic diversity in Plasmodium falciparum merozoite surface protein 1 and 2 coding genes and its implications in malaria epidemiology: a review of published studies from 1997-2007. J Vector Borne Dis. 2009;46(1):1-12.
19. Andolina C, Rek JC, Briggs J, Okoth J, Musiime A, Ramjith J, *et al*. Sources of persistent malaria transmission in a setting with effective malaria control in eastern Uganda: a longitudinal, observational cohort study. Lancet Infect Dis. 2021;21(11):1568-78. doi:10.1016/S1473-3099(21)00072-4
20. Sumner KM, Freedman E, Abel L, Obala A, Pence BW, Wesolowski A, *et al*. Genotyping cognate Plasmodium falciparum in humans and mosquitoes to estimate onward transmission of asymptomatic infections. Nat Commun. 2021;12:909. doi:10.1038/s41467-021-21269-2
21. Lapp Z, Obala AA, Abel L, Rasmussen DA, Sumner KM, Freedman E, *et al*. Plasmodium falciparum genetic diversity in coincident human and mosquito hosts. mBio. 2022;13(3):e0227721. doi:10.1128/mbio.02277-21
22. Biabi MFAB, Fogang B, Essangui E, Maloba F, Donkeu C, Keumoe R, *et al*. High prevalence of polyclonal Plasmodium falciparum infections and association with poor IgG antibody responses in a hyper-endemic area in Cameroon. Trop Med Infect Dis. 2023;8(8):390. doi:10.3390/tropicalmed8080390
23. Yakubu B, Longdet IY, Tony HJ, Davou DT, Obishakin E. High-complexity Plasmodium falciparum infections, North Central Nigeria, 2015-2018. Emerg Infect Dis. 2019;25(7):1330-7. doi:10.3201/eid2507.181614
24. Markers for the molecular epidemiology of P. vivax: microsatellites and antigen-encoding genes. Malar J. 2015;14:84. doi:10.1186/s12936-015-0846-5
25. Amplicon deep sequencing improves detection of minority clones in malaria infections. Malar J. 2018;17:437. doi:10.1186/s12936-018-2337-y
26. Lopez L, Koepfli C. Systematic review of Plasmodium falciparum and Plasmodium vivax polyclonal infections: impact of prevalence, study population characteristics, and laboratory procedures. PLoS One. 2021;16(6):e0249382. doi:10.1371/journal.pone.0249382
27. The multiplicity of P. falciparum infections is associated with acquired immunity to asexual blood-stage antigens in Gabonese children. Infect Immun. 2008;76(5):2066-74. doi:10.1128/IAI.01522-07
28. Risk of clinical malaria from asymptomatic multiclonal infections in children declines with age. Clin Infect Dis. 2019;69(12):2127-34. doi:10.1093/cid/ciz105
29. Preferential transmission of minority and drug-resistant clones in polyclonal infections in Mali. Malar J. 2025;24:298. doi:10.1186/s12936-025-05298-6
30. Antigenic diversity and immune evasion in malaria parasites. Trends Parasitol. 2012;28(5):214-23. doi:10.1016/j.pt.2012.02.005
31. Microsatellites reveal high polymorphism in P. falciparum in mainland Tanzania. Malar J. 2024;23:401. doi:10.1186/s12936-024-04901-6
32. P. falciparum merozoite surface protein 2 genetic polymorphism and multiplicity in Northwest Ethiopia. BMC Infect Dis. 2025;25:676. doi:10.1186/s12879-025-10676-1
33. Olasehinde GI, Diji-Geske RI, Fadina I, Arogundade D, Darby P, Adeleke A, *et al*. Epidemiology of Plasmodium falciparum infection and drug resistance markers in Ota Area, Southwestern Nigeria. Infect Drug Resist. 2019;12:1941-9. doi:10.2147/IDR.S190703
34. Kathryn JW, Sachel M, David AF. Molecular mechanisms of drug resistance in Plasmodium falciparum malaria. Annu Rev Microbiol. 2020;74:431-54. doi:10.1146/annurev-micro-020518-115546
35. World Health Organization. Guidelines for the treatment of malaria. 2nd ed. Geneva: World Health Organization; 2010.
36. Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois AC, Khim N, *et al*. A molecular marker of artemisinin-resistant Plasmodium falciparum malaria. Nature. 2014;505(7481):50-5. doi:10.1038/nature12876
37. Greenwood B, Mutabingwa T. Malaria in 2002. Nature. 2002;415(6872):670-2. doi:10.1038/415670a
38. Pulcini S, Staines HM, Pittman JK, Slavic K, Doerig C, Halbert J, *et al*. Expression in yeast links field polymorphisms in PfATP6 to *in vitro* artemisinin resistance and identifies new inhibitor classes. J Infect Dis. 2013;208(3):468-78. doi:10.1093/infdis/jit171
39. Straimer J, Gnadig NF, Witkowski B, Amaratunga C, Duru V, Ramadani AP, *et al*. K13-propeller mutations confer artemisinin resistance in Plasmodium falciparum clinical isolates. Science. 2015;347(6220):428-31. doi:10.1126/science.1260867
40. World Health Organization. Antimalarial drug combination therapy: report of a WHO technical consultation. Geneva: World Health Organization; 2001.
41. Shafik SH, Dhingra SK, Hott A, *et al*. Mechanistic basis

for multidrug resistance and collateral drug sensitivity in Plasmodium falciparum. PLoS Biol. 2022;20(1):e3001616. doi:10.1371/journal.pbio.3001616
42. Ochong EO, van den Broek IVF, Keus K, Nzila A. Short report: association between chloroquine and amodiaquine resistance and allelic variation in the Plasmodium falciparum multiple drug resistance 1 gene and the chloroquine resistance transporter gene in isolates from southern Sudan. Am J Trop Med Hyg. 2003;69(2):141-4.
43. Duvalsaint M, Conrad MD, Tukwasibwe S, Tumwebaze PK, Legac J, Cooper RA, *et al*. Balanced impacts of fitness and drug pressure on the evolution of PfMDR1 polymorphisms in Plasmodium falciparum. Malar J. 2021;20(1):292. doi:10.1186/s12936-021-03823
44. Ochong E, Tumwebaze PK, Byaruhanga O, Greenhouse B, Rosenthal PJ. Fitness consequences of Plasmodium falciparum pfmdr1 polymorphisms inferred from ex vivo culture of Ugandan parasites. Antimicrob Agents Chemother. 2013;57(9):4245-51. doi:10.1128/AAC.00186-13
45. Klein EY, Smith DL, Laxminarayan R, Levin S. Superinfection and the evolution of resistance to antimalarial drugs. Proc Biol Sci. 2012;279(1743):3834-42. doi:10.1098/rspb.2012.1064
46. Watson OJ, Gao B, Nguyen TD, Tran TN, White LJ, Dondorp AM, *et al*. Pre-existing partner-drug resistance facilitates the emergence and spread of artemisinin resistance: a consensus modelling study [Preprint]. bioRxiv. 2021 [cited 2021 Apr 25]. Available from: https://www.biorxiv.org/content/10.1101/2021.04.08.437876v1
47. Laufer MK, Thesing PC, Eddington ND, Masonga R, Dzinjalamala FK, Takala SL, *et al*. Return of chloroquine antimalarial efficacy in Malawi. N Engl J Med. 2006;355(19):1959-66. doi:10.1056/NEJMoa062032
48. Lu F, Zhang M, Culleton RL, Xu S, Tang J, Zhou H, *et al*. Return of chloroquine sensitivity to Africa? Surveillance of African Plasmodium falciparum chloroquine resistance through malaria imported to China. Parasit Vectors. 2017;10:355. doi:10.1186/s13071-017-2276-5
49. Okell LC, Reiter LM, Ebbe LS, Baraka V, Bisanzio D, Watson OJ, *et al*. Emerging implications of policies on malaria treatment: genetic changes in the Pfmdr-1 gene affecting susceptibility to artemether-lumefantrine and artesunate-amodiaquine in Africa. BMJ Glob Health. 2018;3(5):e000999. doi:10.1136/bmjgh-2018-000999
50. Clyde DF, Shute GT. Resistance of Plasmodium falciparum in Tanganyika to pyrimethamine administered at weekly intervals. Trans R Soc Trop Med Hyg. 1957;51(6):505-13. doi:10.1016/0035-9203(57)90039-9
51. Peterson DS, Walliker D, Wellems TE. Evidence that a point mutation in dihydrofolate reductase-thymidylate synthase confers resistance to pyrimethamine in falciparum malaria. Proc Natl Acad Sci U S A. 1988;85(23):9114-8. doi:10.1073/pnas.85.23.9114
52. Plowe CV, Kublin JG, Doumbo OK. P. falciparum dihydrofolate reductase and dihydropteroate synthase mutations: epidemiology and role in clinical resistance to antifolates. Drug Resist Updat. 1998;1(6):389-96. doi:10.1016/S1368-7646(98)80014-9
53. Kublin JG, Dzinjalamala FK, Kamwendo DD, Malkin EM, Cortese JF, Martino LM, *et al*. Molecular markers for failure of sulfadoxine-pyrimethamine and chlorproguanil-dapsone treatment of Plasmodium falciparum malaria. J Infect Dis. 2002;185(3):380-8. doi:10.1086/338566
54. Clyde DF. Genesis of chloroquine-resistant Plasmodium falciparum in the American region. Med Trop Cooperaz Sviluppo. 1987;3:41-4.
55. Fidock DA, Nomura T, Talley AK, Cooper RA, Dzekunov SM, Ferdig MT, *et al*. Mutations in the P. falciparum digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. Mol Cell. 2000;6(4):861-71. doi:10.1016/S1097-2765(05)00083-6
56. Wootton JC, Feng X, Ferdig MT, Cooper RA, Mu J, Baruch DI, *et al*. Genetic diversity and chloroquine selective sweeps in Plasmodium falciparum. Nature. 2002;418(6895):320-3. doi:10.1038/nature00813
57. Doumbo OK, Kayentao K, Djimde A, Cortese JF, Diourte Y, Konaré A, *et al*. Rapid selection of Plasmodium falciparum dihydrofolate reductase mutants by pyrimethamine prophylaxis. J Infect Dis. 2000;182(3):993-6. doi:10.1086/315791
58. Cortese JF, Caraballo A, Contreras CE, Plowe CV. Origin and dissemination of Plasmodium falciparum drug-resistance mutations in South America. J Infect Dis. 2002;186(7):999-1006. doi:10.1086/342946
59. Roper C, Pearce R, Nair S, Sharp B, Nosten F, Anderson T. Intercontinental spread of pyrimethamine-resistant malaria. Science. 2004;305(5687):1124. doi:10.1126/science.1098876
60. Kublin JG, Cortese JF, Njunju EM, Mukadam RA, Wirima JJ, Kazembe PN, *et al*. Reemergence of chloroquine-sensitive Plasmodium falciparum malaria after cessation of chloroquine use in Malawi. J Infect Dis. 2003;187(12):1870-5. doi:10.1086/375419
61. Laufer MK, Thesing PC, Eddington ND, Masonga R, Dzinjalamala FK, Takala SL, *et al*. Return of chloroquine antimalarial efficacy in Malawi. N Engl J Med. 2006;355(19):1959-66. doi:10.1056/NEJMoa062032
62. Alker AP, Lim P, Sem R, Shah NK, Yi P, Bouth DM, *et al*. Pfmdr1 and *in vivo* resistance to artesunate-mefloquine in falciparum malaria on the Cambodian-Thai border. Am J Trop Med Hyg. 2007;76(4):641-7. doi:10.4269/ajtmh.2007.76.641
63. Laufer MK, Djimde AA, Plowe CV. Monitoring and deterring drug-resistant malaria in the era of combination therapy. Am J Trop Med Hyg. 2007;77(6 Suppl):160-9.
64. Nayyar GML, Breman JG, Newton PN, Herrington J. Poor-quality antimalarial drugs in southeast Asia and sub-Saharan Africa. Lancet Infect Dis. 2012;12(6):488-96. doi:10.1016/S1473-3099(12)70064-6
65. Tabernero P, Fernández FM, Green M, Guerin PJ, Newton PN. Mind the gaps - the epidemiology of poor-quality anti-malarials in the malarious world - analysis of the WorldWide Antimalarial Resistance Network database. Malar J. 2014;13:139. doi:10.1186/1475-2875-13-139
66. Okeke TA, Uzochukwu BSC, Okafor HU. An in-depth study of patent medicine sellers' perspectives on malaria in a rural Nigerian community. Malar J. 2006;5:97.

doi:10.1186/1475-2875-5-97
67. Onwujekwe O, Obikeze E, Uzochukwu B, Okoronkwo I, Onwughalu B. Improving equity in malaria treatment: relationship of socio-economic status with health seeking as well as with perceptions of ease of using the services of different providers for obtaining treatment. Malar J. 2008;7:5. doi:10.1186/1475-2875-7-5
68. Ezenduka CC, Ogbonna BO, Esimone CO. Sources of antimalarial drugs for self-medication in Nigeria: a cross-sectional study of the practice among university students. BMC Public Health. 2014;14:306. doi:10.1186/1471-2458-14-306
69. Bruxvoort K, Goodman C, Kachur SP, Schellenberg D. How patients take malaria treatment: a systematic review of the literature on adherence to antimalarial drugs. PLoS One. 2014;9(1):e84555. doi:10.1371/journal.pone.0084555
70. Ajayi IO, Falade CO, Olley BO, Yusuf B, Gbotosho S, Iyiola TA, *et al*. A qualitative study of the feasibility and community perception on the effectiveness of artemisinin-based combination therapy (ACT) in Ibadan, southwest Nigeria. BMC Health Serv Res. 2008;8:119. doi:10.1186/1472-6963-8-119
71. Ogbonna A, Uneke CJ. Artemisinin-based combination therapy for uncomplicated malaria in sub-Saharan Africa: the efficacy, safety, resistance and policy implementation since Abuja 2000. Trans R Soc Trop Med Hyg. 2008;102(7):621-7. doi:10.1016/j.trstmh.2008.03.024
72. Federal Ministry of Health Nigeria. National malaria elimination programme (NMEP). National malaria policy 2021–2025. Abuja: Federal Ministry of Health; 2021.
73. Adeneye AK, Jegede AS, Mafe MA, Nwokocha EE. Community perceptions and home management of malaria in selected rural communities of Ogun State, Nigeria. Int Q Community Health Educ. 2013;33(1):85-96. doi:10.2190/IQ.33.1.f
74. Wongsrichanalai C, Barcus MJ, Muth S, Sutamihardja A, Wernsdorfer WH. A review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT). Am J Trop Med Hyg. 2007;77(6 Suppl):119-27.
75. Berhane A, Anderson K, Mihreteab S, Gresty K, Rogier E, Mohamed S, *et al*. Major threat to malaria control programs by Plasmodium falciparum lacking histidine-rich protein 2, Eritrea. Emerg Infect Dis. 2018;24(3):462-70. doi:10.3201/eid2403.171723
76. Nwakanma DC, Duffy CW, Amambua-Ngwa A, Oriero EC, Gomez-Escobar N, Kwiatkowski DP, *et al*. Changes in malaria parasite population structure in The Gambia before and after introduction of ACT. PLoS One. 2014;9(9):e107508. doi:10.1371/journal.pone.0107508
77. Daniels R, Ndiaye D, Wall M, McKinney J, Séne PD, Sabeti PC, *et al*. Rapid, field-deployable method for genotyping and discovery of single-nucleotide polymorphisms associated with drug resistance in Plasmodium falciparum. Antimicrob Agents Chemother. 2012;56(6):2976-86. doi:10.1128/AAC.05737-11
78. Amambua-Ngwa A, Tetteh KK, Manske M, Gomez-Escobar N, Stewart LB, Deerhake ME, *et al*. Population genomic scan for candidate signatures of balancing selection to guide antigen characterization in malaria parasites. PLoS Genet. 2012;8(11):e1002992. doi:10.1371/journal.pgen.1002992
79. Adebayo JO, Krettli AU. Potential antimalarials from Nigerian plants: a review. J Ethnopharmacol. 2011;133(2):289-302. doi:10.1016/j.jep.2010.11.024
80. Huijben S, Bell AS, Sim DG, Tomasello D, Mideo N, Day T, *et al*. Aggressive chemotherapy and the selection of drug-resistant pathogens. PLoS Pathog. 2013;9(9):e1003578. doi:10.1371/journal.ppat.1003578
81. Stepniewska K, Ashley E, Lee SJ, Annerberg A, Yeung S, Cheah PY, *et al*. *In vivo* parasitological measures of artemisinin resistance in Plasmodium falciparum. Malar J. 2010;9:48. doi:10.1186/1475-2875-9-48
82. Collins WE, Jeffery GM. A retrospective examination of reinfection of humans with malaria parasites. Am J Trop Med Hyg. 1999;61(1 Suppl):20-35. doi:10.4269/ajtmh.1999.61.20
83. Snounou G, Beck HP. The use of PCR genotyping in the assessment of recrudescence or reinfection after antimalarial drug treatment. Parasitol Today. 1998;14(11):462-7. doi:10.1016/S0169-4758(98)01339-0
84. Gadalla NB, Adam I, Elzaki SE, Abdelrahim S, El-Souki M, Ahmed A, *et al*. Increased multiplicity of infection and prevalence of drug resistance in Plasmodium falciparum isolates from Sudan. Am J Trop Med Hyg. 2011;85(5):873-9. doi:10.4269/ajtmh.2011.11-0194
85. Desai M, ter Kuile FO, Nosten F, McGready R, Asamoa K, Brabin B, *et al*. Epidemiology and burden of malaria in pregnancy. Lancet Infect Dis. 2007;7(2):93-104. doi:10.1016/S1473-3099(07)70021-X
86. White NJ. Does antimalarial mass drug administration increase or decrease the risk of resistance? Lancet Infect Dis. 2017;17(1):e15-20. doi:10.1016/S1473-3099(16)30269-7
87. Nkhoma SC, Nair S, Cheeseman IH, Rohr-Allegrini C, Singlam S, Nosten F, *et al*. Close kinship within multiple-genotype malaria parasite infections. Proc Biol Sci. 2012;279(1738):2589-98. doi:10.1098/rspb.2011.2396
88. Babiker HA, Ranford-Cartwright LC, Walliker D. Genetic structure and dynamics of Plasmodium falciparum infections in the Kilombero region of Tanzania. Trans R Soc Trop Med Hyg. 1999;93(Suppl 1):11-4. doi:10.1016/S0035-9203(99)90316-8
89. Lindblade KA, Steinhardt L, Samuels A, Kachur SP, Slutsker L. The silent threat: asymptomatic parasitemia and malaria transmission. Expert Rev Anti Infect Ther. 2013;11(6):623-39. doi:10.1586/eri.13.45
90. Babiker HA, Gadalla AA, Ranford-Cartwright LC. The role of asymptomatic Plasmodium falciparum infections in the spread of antimalarial drug resistance. Trends Parasitol. 2013;29(9):394-402. doi:10.1016/j.pt.2013.06.006
91. Tiono AB, Ouédraogo A, Ogutu B, Diarra A, Coulibaly S, Gansané A, *et al*. A controlled, parallel, cluster-randomized trial of community-wide screening and treatment of asymptomatic carriers of Plasmodium falciparum in Burkina Faso. Malar J. 2013;12:79. doi:10.1186/1475-2875-12-79
92. Ariey F, Witkowski B, Amaratunga C, Beghain J, Langlois AC, Khim N, *et al*. A molecular marker of artemisinin-resistant Plasmodium falciparum malaria. Nature. 2014;505(7481):50-5. doi:10.1038/nature12876
93. World Health Organization. Artemisinin resistance and artemisinin-based combination therapy efficacy.

Geneva: World Health Organization; 2018.
94. Mwingira F, Nkwengulila G, Schoepflin S, Sumari D, Beck HP, Felger I, *et al*. Plasmodium falciparum msp1, msp2 and glurp allele frequency and diversity in sub-Saharan Africa. Malar J. 2011;10:79. doi:10.1186/1475-2875-10-79
95. Färnert A. Plasmodium falciparum population dynamics: only snapshots in time? Trends Parasitol. 2008;24(8):340-4. doi:10.1016/j.pt.2008.04.008
96. Amambua-Ngwa A, Tetteh KK, Manske M, Gomez-Escobar N, Stewart LB, Deerhake ME, *et al*. Population genomic scan for candidate signatures of balancing selection to guide antigen characterization in malaria parasites. PLoS Genet. 2012;8(11):e1002992. doi:10.1371/journal.pgen.1002992
97. Wongsrichanalai C, Barcus MJ, Muth S, Sutamihardja A, Wernsdorfer WH. A review of malaria diagnostic tools: microscopy and rapid diagnostic test (RDT). Am J Trop Med Hyg. 2007;77(6 Suppl):119-27.
98. World Health Organization. Malaria rapid diagnostic test performance. Geneva: World Health Organization; 2021.
99. Polley SD, González IJ, Mohamed D, Daly R, Bowers K, Watson J, *et al*. Clinical evaluation of loop-mediated isothermal amplification for detection of malaria in patient samples. J Clin Microbiol. 2013;51(9):2919-23. doi:10.1128/JCM.00860-13
100. Myhrvold C, Freije CA, Gootenberg JS, Abudayyeh OO, Metsky HC, Durbin AF, *et al*. Field-deployable viral diagnostics using CRISPR-Cas13. Science. 2018;360(6387):444-8. doi:10.1126/science.aas8836
101. Bruxvoort K, Goodman C, Kachur SP, Schellenberg D. How patients take malaria treatment: a systematic review of the literature on adherence to antimalarial drugs. PLoS One. 2014;9(1):e84555. doi:10.1371/journal.pone.0084555
102. Uzochukwu BS, Onwujekwe OE, Ezumah NN, Obikeze EN. Improving rational treatment of malaria: perceptions and influence of RDTs on prescribing behaviour of health workers in southeast Nigeria. PLoS One. 2011;6(1):e14627. doi:10.1371/journal.pone.0014627
103. van der Pluijm RW, Imwong M, Chau NH, Hoa NT, Thuy-Nhien NT, Thanh NV, *et al*. Triple artemisinin-based combination therapies versus artemisinin-based combination therapies for uncomplicated Plasmodium falciparum malaria: a multicentre, open-label, randomised clinical trial. Lancet. 2020;395(10233):1345-60. doi:10.1016/S0140-6736(20)30552-3
104. Pongtavornpinyo W, Yeung S, Hastings IM, Dondorp AM, Day NP, White NJ. Spread of antimalarial drug resistance: mathematical model with implications for ACT policy. Malar J. 2008;7:229. doi:10.1186/1475-2875-7-229
105. World Health Organization. Global plan for insecticide resistance management in malaria vectors. Geneva: World Health Organization; 2012.
106. Ranson H, N'Guessan R, Lines J, Moiroux N, Nkuni Z, Corbel V. Pyrethroid resistance in African anopheline mosquitoes: what are the implications for malaria control? Trends Parasitol. 2011;27(2):91-8. doi:10.1016/j.pt.2010.08.004
107. Gleave K, Lissenden N, Richardson M, Choi L, Ranson H. Piperonyl butoxide (PBO) combined with pyrethroids in insecticide-treated nets to prevent malaria in Africa. Cochrane Database Syst Rev. 2018;11:CD012776. doi:10.1002/14651858.CD012776.pub2
108. Njoroge M, Sitati A, Mundia CW, Mutai P, Ongeri B, Muriuki JM, *et al*. Community knowledge and perceptions on malaria prevention and control in a malaria endemic area of western Kenya. BMC Public Health. 2020;20(1):1191. doi:10.1186/s12889-020-09295-6
109. Tiono AB, Ouédraogo A, Ogutu B, Diarra A, Coulibaly S, Gansané A, *et al*. A controlled, parallel, cluster-randomized trial of community-wide screening and treatment of asymptomatic carriers of Plasmodium falciparum in Burkina Faso. Malar J. 2013;12:79. doi:10.1186/1475-2875-12-79
110. Cowell AN, Winzeler EA. The genomic architecture of antimalarial drug resistance. Brief Funct Genomics. 2019;18(5):314-28. doi:10.1093/bfgp/elz008
111. Nair S, Li X, Arya GA, McDew-White M, Ferrari M, Nosten F, *et al*. Fitness costs and the rapid spread of kelch13-C580Y substitutions conferring artemisinin resistance. Antimicrob Agents Chemother. 2018;62(9):e00605-18. doi:10.1128/AAC.00605-18
112. Tessema SK, Hathaway NJ, Teyssier NB, Murphy M, Chen A, Aydemir O, *et al*. Sensitive, highly multiplexed sequencing of microhaplotypes from the Plasmodium falciparum heterozygome. J Infect Dis. 2020;221(9):1491-503. doi:10.1093/infdis/jiz645
113. Balikagala B, Mita T, Ikeda M, Sakurai M, Yatsushiro S, Takahashi N, *et al*. Absence of *in vivo* selection for kelch13 C580Y mutant parasites after artemether-lumefantrine treatment in Uganda. Malar J. 2022;21(1):182. doi:10.1186/s12936-022-04194-0
114. World Health Organization. Global technical strategy for malaria 2016–2030, 2021 update. Geneva: World Health Organization; 2021.
115. Conrad MD, Rosenthal PJ. Antimalarial drug resistance in Africa: the calm before the storm? Lancet Infect Dis. 2019;19(10):e338-51. doi:10.1016/S1473-3099(19)30261-0