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INFECTIOUS DISEASE EPIDEMIOLOGY (M YOTEBIENG, SECTION EDITOR)

Antimalarial Drug Resistance and Implications for the WHO Global Technical Strategy

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Abstract

Purpose of Review Five years have passed since the World Health Organization released its Global Technical Strategy for Malaria (GTS). In that time, progress against malaria has plateaued. This review focuses on the implications of antimalarial drug resistance for the GTS and how interim progress in parasite genomics and antimalarial pharmacology offer a bulwark against it.

Recent Findings For the first time, drug resistance–conferring genes have been identified and validated before their global expansion in malaria parasite populations. More efficient methods for their detection and elaboration have been developed, although low-density infections and polyclonality remain a nuisance to be solved. Clinical trials of alternative regimens for multidrug-resistant malaria have delivered promising results. New agents continue down the development pipeline, while a nascent infrastructure in sub-Saharan Africa for conducting phase I trials and trials of transmission-blocking agents has come to fruition after years of preparation.

Summary These and other developments can help inform the GTS as the world looks ahead to the next two decades of its implementation. To remain ahead of the threat that drug resistance poses, wider application of genomic-based surveillance and optimization of existing and forthcoming antimalarial drugs are essential.

Keywords Malaria · *Plasmodium* · Drug resistance · World Health Organization Global Technical Strategy for Malaria

Introduction

Malaria stubbornly persists in tropical and subtropical regions of the world despite concerted global efforts that date back to the World Health Organization (WHO) Global Malaria Eradication Programme in the 1950s and 1960s [1, 2]. The program largely excluded sub-Saharan Africa, and its ultimate abandonment was driven by a host of factors including, in part, the emergence and spread of drug resistance to chloroquine (CQ) and related 4-aminoquinolines in use at the time [3]. Over half a century later, the emergence of antimalarial

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drug resistance remains an ever-present threat to global malaria control.

In 2016, the WHO reaffirmed its vision of global malaria eradication and provided a technical framework to guide local, national, and regional efforts outlined in the Global Technical Strategy for Malaria 2016-2030 [4]. The strategy centers on universal access to malaria testing and treatment, acceleration toward elimination where feasible, enhanced surveillance, continued research and innovation, and infrastructural and capacity-building investments. Soberly, it acknowledges the imposing challenges to malaria control, manifest in the most recent World Malaria Report that documented stalling and, in certain areas ranging from South America to sub-Saharan Africa, reversing progress [5]. The Global Technical Strategy set a milestone of achieving a 40% reduction in malaria mortality and case incidence by 2020 and a 90% reduction by 2030 [4]. The latest data suggests that these first milestones will not be met. In response, the WHO updated its agenda to increase focus on high-burden areas [5, 6]. Meanwhile, the current coronavirus pandemic is anticipated to further harm malaria control prospects [7•, 8•].

Human malaria is caused by one of five *Plasmodium* spp. of which *P. falciparum* and *P. vivax* are the most prevalent. While *P. vivax* has a wider geographic range and a biology that poses unique challenges to eradication, *P. falciparum* is responsible for the vast majority of malaria-attributable deaths and is the predominant species in sub-Saharan Africa, which bears > 90% of the global malaria burden [5]. The other human-infecting species, including *P. ovale*, *P. malariae*, and the zoonotic *P. knowlesi*, are less prevalent, generally less lethal (with the exception of *P. knowlesi*) and less studied in terms of drug resistance. This review therefore focuses primarily on drug resistance in *P. falciparum*, with additional discussion of *P. vivax*.

Malaria, with few exceptions, is treated with combination therapy (Table 1). Artemisinin-based combination therapies (ACTs) are the current first-line agents for curative treatment [9]. A complete course of ACT is also given in severe malaria following induction with intravenous artesunate [9–11]. They combine short-acting but highly potent artemisinin derivatives with less potent, longer-acting agents. The coformulation of agents with mismatched pharmacokinetics, while not unique to antimalarial therapy, is expected to impede the development of artemisinin resistance but comes with the potential expense of promoting resistance to the partner agents which linger for weeks to months at subtherapeutic concentrations [12]. Pharmacologic autoinduction of artemisinins, whereby the compounds upregulate their own metabolism with repeated dosing, may also help promote resistance by exposing parasites to subtherapeutic concentrations over the treatment course [13].

Among threats to malaria control, the emergence and spread of drug-resistant parasites require a response that leverages innovations in parasite genomics, drug development, and multinational collaborations to identify, track, contain, and treat multidrug-resistant malaria (Table 2) [14, 15]. Here, we review and discuss the historical origins and spread of drug-resistant malaria and their impacts on past control efforts, a brief overview of mechanisms of resistance, methods of drug resistance surveillance, the effects of resistance on treatment and prevention, and how innovations in parasite genomics and drug development can advance the Global Technical Strategy.

Historical Origins and Present Distribution of Drug-Resistant Malaria

Malaria parasite resistance to essentially all currently and previously available antimalarial drugs has arisen multiple times throughout the course of the last century and more. Quinine was the first commercial antimalarial, extracted from the bark of the South American cinchona tree and first traded in Europe in the seventeenth century. By the turn of the twentieth century, quininization, the mass administration of quinine or its relatives, was carried out in large tea estates, sugar and rubber plantations, and similar settings where malaria-naïve workers migrated to malarious areas [16–18]. Early reports of suspected quinine resistance emerged as early as 1910 [19]. Quinine and its relatives were then commonly combined with the early 8-aminoquinolines, forebears to primaquine and tafenoquine, but combination therapy was replaced by CQ monotherapy with the drug's advent during World War II.

In the middle of the WHO Global Malaria Eradication Programme (1955–1969), the first reports of CQ-resistant *P. falciparum* emerged independently in South America and Southeast Asia [20, 21]. Within two decades, CQ-resistant *P. falciparum* swept across the tropical and subtropical world [22]. CQ was gradually replaced by sulfadoxine-pyrimethamine (SP), but resistance to SP appeared rapidly after its introduction [23–25]. The emergence of drug-resistant *P. falciparum* in sub-Saharan Africa was devastating, with doubling to tripling of case incidence and mortality (Fig. 1) [30]. As alternative agents were introduced—proguanil, amodiaquine, mefloquine, piperaquine, atovaquone—the identification of drug-resistant parasites followed closely [31]. Malaria resurged worldwide, and the measurable progress that had been made against malaria during the eradication effort was eroded, compounded by delays in replacing CQ and SP on national formularies with ACTs [32].

After ACTs were introduced in the late 1990s and early 2000s, initial reports of partial artemisinin and ACT resistance appeared in Southeast Asia [33, 34] and were followed by frank treatment failures of artesunate-mefloquine (AS-MQ) and later dihydroartemisinin-piperaquine (DHA-PPQ) due to phenotype- and genotype-confirmed resistance to both the

Table 1 *P. falciparum* blood schizonticides currently in use and their associated genetic markers of resistance

Drug class and agent	Available coformulations	Timeline of resistance		Molecular markers of resistance	
		Introduction of agent	First report of resistance	Implicated genes	Polymorphism or variant
4-Aminoquinolines					
Chloroquine	CQ monotherapy	1945	1957	*pfcrt*	SNPs
				pfmdr1	SNPs
Amodiaquine	AS-AQ	1951	1971	*pfcrt*	SNPs
				pfmdr1	SNPs
Arylamino alcohols					
Lumefantrine	AM-LF	1992[c]	-[a]	*pfcrt*[b]	SNPs[b]
				pfmdr1[b]	Copy # variant[b]
Mefloquine	MQ monotherapy, AS-MQ	1977	1982	*pfmdr1*	Copy # variant
Quinine	QN monotherapy	1632	1910	*pfmdr1*	SNPs
				pfmdr6	SNPs
				pfcrt	SNPs
				pfmrp1	SNPs
				pfnhe1	SNPs
Mannich base					
Pyronaridine	AS-PY	2014	-[a]	*pfcrt*[b]	SNPs[b]
Artemisinins					
Artemether	AM-LF	1992[c]	-[a]	*pfk13*	SNPs
Artesunate	AS monotherapy, AS-SP, AS-MQ, AS-PY	1978	2002[d] 2011[e]	*pfk13*	SNPs
Dihydroartemisinin	DHA-PPQ	1997[e]	2010[f]	*pfk13*	SNPs
Bisquinoline					
Piperaquine	DHA-PPQ	"	"	*pfpm2*	Copy # variant
				pfcrt	SNPs
Naphthoquinone					
Atovaquone	ATQ-PGL	1996	1997[g]	*pfcytb*	SNPs
Antifolates					
Proguanil	ATQ-PGL	1948	1949[h]	*pfdhfr*	SNPs
Pyrimethamine	SP	1967	1967[i]	*pfdhfr*	SNPs
Sulfadoxine	SP	"	"	*pfdhps*	SNPs

AM artemether, *AQ* amodiaquine, *AS* artesunate, *ATQ* atovaquone, *CQ* chloroquine, *DHA* dihydroartemisinin, *LF* lumefantrine, *MQ* mefloquine, *P* pyrimethamine, *PGL* proguanil, *PPQ* piperaquine, *PY* pyronaridine, *QN* quinine, *S* sulfadoxine, *SNPs* single nucleotide polymorphisms

[a] No confirmed resistance

[b] From in vitro studies of experimentally generated resistance, unconfirmed role in field isolates

[c] Introduction of the combination AM-LF

[d] Date applies to the combination AS-MQ

[e] Date applies to the combination AS-SP

[f] Date applies to the combination DHA-PPQ

[g] Resistance to ATQ alone; resistance to the combination ATQ-PGL was detected in 2002

[h] Resistance to PGL alone; see note above

[i] Date applies to the combination SP

artemisinin derivates and partner drugs [35••, 36–44]. Treatment failures well exceeded the threshold of 10% typical for enacting policy change [41]. Drug efficacy studies of the other ACTs have variably found therapeutic failures > 10%, but these studies were limited by small sample sizes and lack of phenotypic and/or genotypic correlates of resistance

Table 2 Policy prescriptions for confronting antimalarial drug resistance using parasite genomics and clinical pharmacology

	Parasite genomics	Antimalarial drug evaluation	Antimalarial pharmacology
Innovation	Identification and validation of new molecular markers and mechanisms of resistance Solutions for genotyping low parasitemia infections and polyclonality Development of low-cost genotyping assays with genetic resolution for tracking parasite importation	Establishing methods for continuous culture of *P. vivax* Development of in vitro models for evaluating antimalarial efficacy for non-falciparum species Development of low-cost and simple methods for monitoring drug quality	Discovery and development of novel pharmacophores, classes, and mechanisms of action Updated paradigms of parasite clearance and other pharmaco-dynamic parameters of drug efficacy
Optimization	Streamlining genomic techniques to enhance accessibility Expansion of tools for assessing the genetics of polyclonal infections	Strengthening regional collaborations for resistance testing and surveillance	Dose optimization in pediatric and pregnant populations earlier in drug development Broaden uptake of transmission-blocking 8-aminoquinolines (e.g., single low-dose primaquine) Retailor ACT posology and formulation to prolong their utility (e.g., 6-day regimens, sequential ACT, triple ACT)
Investment	Expansion of sequencing infrastructure in sub-Saharan Africa Training in bioinformatics and data analysis Development of data visualization tools for genomic data for policy makers	Expansion of infrastructure for ex vivo and in vitro antimalarial resistance testing in sub-Saharan Africa Expansion of infrastructure for monitoring drug quality	Support of phase 1 and 2 clinical trials in endemic or endemic-adjacent areas Instrumentation and human upskilling for biochemical analysis Investment in pharmacovigilance systems in endemic countries

ACT artemisinin-based combination therapy

[45–48]. One exception is resistance to artesunate-sulfadoxine-pyrimethamine (AS-SP) reported in areas of high preexisting antifolate resistance; antifolate resistance effectively reduces AS-SP to artemisinin monotherapy, which is known to fail in up to 50% of cases due in part to its rapid elimination and autoinduction with repeated doses [13, 49].

Antimalarial resistance in non-falciparum species has been slower to emerge, thought due to lower parasite numbers in the human host and hence fewer mutation events, and, for *P. vivax* and *P. ovale*, the ability to evade blood schizonticides through forming hypnozoites in the liver. In the late 1980s, CQ resistance in *P. vivax* was first reported in non-immune Australian travels to Papua New Guinea, and by the early 2000s, CQ resistance was also documented in *P. malariae* [50–52]. Genetic signatures of *P. vivax* worldwide suggest that, today, CQ resistance has expanded globally [53].

Today, the distribution of drug-resistant *P. falciparum* remains variable across the globe, reflecting in part patterns in drug deployment and transmission intensity. Resistance to two of the ACTs, DHA-PPQ and AS-MQ, is well documented in Southeast Asia [33, 40]. Reports of ACT treatment failures in malaria-naïve travelers who contracted *P. falciparum* malaria in sub-Saharan Africa echo the first reports of CQ-resistant malaria, though none contain definitive confirmation of resistance and at least two of the reports included failures attributable to subtherapeutic dosing rather than drug resistance [54–56, 57•, 58, 59]. Evidence of genotypic and phenotypic correlates of resistance to one or more ACT components is beginning to emerge in sub-Saharan Africa, South Asia, and South America including a recent report from Rwanda where *P. falciparum kelch13* (*pfk13*) R561H, P574L, and C469Y alleles—previously linked to a delayed clearance phenotype [60•]—were detected [33, 40, 61••, 62•, 63•]. Resistance to the antifolates remains widespread, while reversion of CQ-resistant parasite populations to the CQ-susceptible wild type followed in the wake of withdrawing CQ from national formularies in eastern and central-southern Africa [64–71].

Mechanisms of Antimalarial Resistance

At the molecular level, the emergence and propagation of drug-resistant *Plasmodium* spp. are intrinsically tied to the diverse forms assumed by the parasite and the variety of environments it traverses, from the mosquito midgut and salivary glands to human hepatocytes and erythrocytes [31]. The malaria parasite spends most of its life in a haploid state, only briefly diploid during sexual recombination in mosquitoes [72]. The propagation of drug resistance through a parasite population requires mutant parasites to successfully undergo gametocytogenesis and sporogony, two of the parasite lifecycle bottlenecks. Mutations that hamper these or other vital functions will propagate poorly or not at all, as with atovaquone resistance–conferring mutations in the *Plasmodium* cytochrome b complex which appear to render the parasite intransmissible [73].

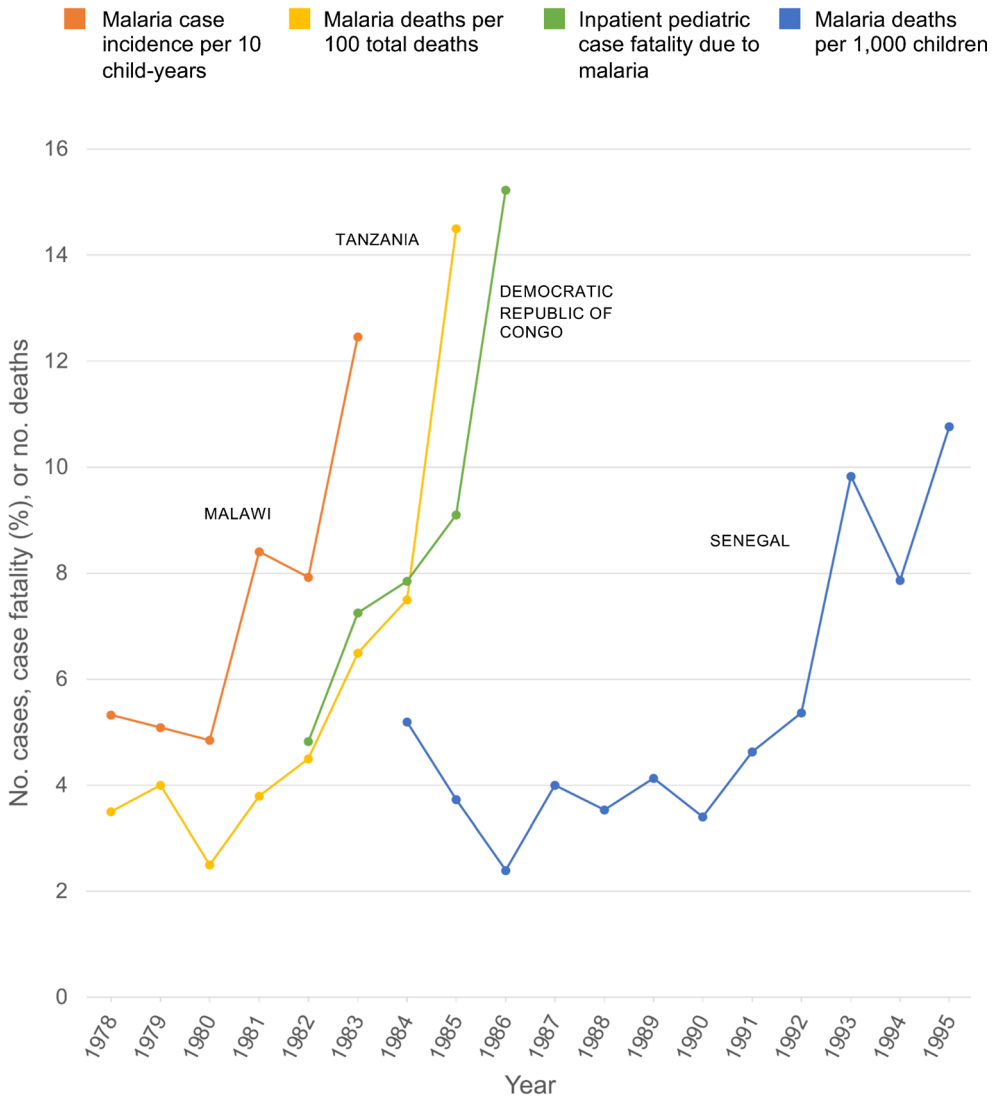

Fig. 1 Trends in malaria case incidence, deaths, and case fatality after the introduction and spread of chloroquine-resistant malaria into sub-Saharan Africa. Data are from four historical studies [26•, 27–29]

Comprehensive reviews of the mechanisms and genotypic markers of antimalarial drug resistance were recently published elsewhere by Conrad and Ross [74, 75•]. Mechanisms of resistance in malaria parasites include similar mechanisms to those described in other microorganisms (e.g., drug efflux, alteration to the drug target, enzymatic degradation or modification of the drug) as well as less common mechanisms that relate to the parasite's lifecycle and metabolism [76]. For example, mutations in *pfk13* mediate susceptibility to artemisinins by prolonging the time the parasite spends in its earlier, less drug-susceptible ring stage and upregulating the unfolded protein response, essentially arresting development while the artemisinin is rapidly eliminated and artemisinin-damaged peptides are cleared [77, 78•].

Drug resistance in *P. vivax* and *P. malariae* is less well characterized than in *P. falciparum*. In vivax malaria, relapse from hypnozoites complicates the interpretation of treatment outcomes, further made difficult by the current lack of methods for continuous culture of *P. vivax* [52, 79]. Given these challenges, standardized methods for evaluating the efficacy of antimalarials for *P. vivax* are necessary [80]. Historical comparative studies of quinine and chloroquine for vivax malaria support a definition of treatment failure as any recurrent *P. vivax* parasitemia occurring within a certain window of time after treatment (e.g., treatment failure if within 16 days, likely treatment failure if within 17–28 days, possible treatment failure if after 28 days) [81].

Therapeutic failures of CQ for *P. vivax* infection and candidate drug resistance mutations have been documented in most endemic areas [82–91, 92•], prompting some national programs to adopt ACTs as first line for vivax malaria [80]. There are no firmly established molecular markers of drug-resistant *P. vivax*, but population genomic studies of *P. vivax* hint not only at drug pressure from CQ, the first-line *P. vivax* treatment in most countries, but also genetic signatures of possible MQ, antifolate, and artemisinin resistance in areas

of co-endemic *P. falciparum* [53, 93]. There is increasing recognition that mechanisms of drug resistance may differ in substantial ways between different *Plasmodium* spp. While CQ resistance in *P. falciparum* is strongly linked to the K76T mutation in the CQ resistance transporter gene (*pfcrt*) [94, 95], CQ resistance in *P. vivax* appears to be via an unknown mechanism mediated by transcriptional changes in the orthologous *pvcrt-o*, suggesting interspecies differences in the native function of the transporter, which still is unknown [96, 97•, 98, 99].

Drug Resistance Surveillance: Clinical, Phenotypic, and Genotypic Detection

The detection of antimalarial drug resistance relies on clinical, phenotypic, and genotypic data, yet it can be challenging to acquire all three in the resource-deprived settings where malaria is endemic. Current antimalarial resistance surveillance activities therefore often operate using one or two of the three approaches.

Clinical resistance relates to therapeutic failure, wherein a patient experiences persistent or recurrent parasitemia after treatment, and is defined in terms of early or late reappearance of parasites on blood smear and the presence or absence of symptoms [100]. Confirmation of therapeutic failure requires matching the parasite strains from the initial and recurrent episode to distinguish failure from a new infection, which presents sizeable challenges due to multiple-strain (polyclonal) infections, and the often small number of parasites in recurrent episodes [101]. Phenotypic correlates of drug resistance require drug sensitivity testing of in vitro or ex vivo parasite cultures from patients, not currently performed in the course of clinical care nor are the laboratory methods readily available, many of which require specialized assays for individual drugs [102, 103]. Once specific gene mutations are linked to clinical and phenotypic correlates of drug resistance and validated in field-isolated parasites, genotyping parasites for genetic markers of resistance is a third means of surveillance and the most scalable [104].

In practice, drug resistance surveillance has historically been done through therapeutic efficacy studies (TES) of first-line agents that follow a prespecified WHO protocol, recommended every two years in endemic areas [41]. Today, TES commonly combines clinical outcomes with molecular (genotypic) information [105, 106]. In China and Southeast Asia where containment of already-identified ACT resistance is a high priority, protocols for integrated drug efficacy surveillance that incorporates data from imported as well as local cases are under development [107].

The identification and validation of partial artemisinin resistance-conferring mutations in *pfk13* have enabled monitoring for the emergence of artemisinin resistance worldwide [108]. This is the first time a validated molecular marker of antimalarial drug resistance is available prior to its widespread dissemination. There have now been multiple reports of *pfk13* mutations outside of Southeast Asia, though not always accompanied by phenotypic or clinical evidence of drug resistance [61, 109–111]. More recent reports from Guyana, Rwanda, Uganda, and Tanzania have documented validated resistance polymorphisms in *pfk13* [61••, 63•, 112–114, 115•].

Drug Resistance and Malaria Chemotherapy and Chemoprevention

The WHO Global Technical Strategy calls for universalizing access to malaria chemotherapy and chemoprevention, and revisits an old notion of using chemoprophylaxis in endemic populations, an approach historically reserved for short-term use in malaria-naïve travelers to endemic areas [4]. Expanding the use of antimalarials in such ways should go hand in hand with expanding surveillance for antimalarial drug resistance. The Global Technical Strategy outlines a timeline (every 2 years) and threshold of effectiveness (90%) for TES of first-line agents [4]. The strategy also emphasizes the need for expanding the genetic library of molecular markers of drug resistance to facilitate early detection. This is particularly relevant in pre-elimination settings where sustained low-level transmission may be more likely to foster resistant parasites, but low malaria incidence precludes antimalarial efficacy studies [116].

As CQ and SP resistance spread, their replacement by ACTs as first-line treatment for uncomplicated malaria contributed to the regained progress against malaria during the first decade and a half of this century [117]. To help preserve ACT efficacy, several alternatives to conventional ACT regimens have been proposed including sequential double combination ACT, prolonged and/or increased daily frequency of ACT dosing, and triple ACT. Recently, clinical trials of triple ACT have demonstrated their safety and efficacy, including in the treatment of multidrug-resistant malaria (NCT03355664 and NCT02453308) [118, 119]. Co-therapy with a second agent to potentiate parasite killing or reverse drug resistance—such as ACTs or CQ with macrolide antibiotics, or CQ with calcium channel blockers—has also been studied but with unpromising results [120, 121].

The efficacy and durability of ACTs hinge on several factors. Underdosing of some antimalarial drugs in children and pregnant women is thought to have contributed to drug resistance; thus, pediatric formulations and early dose optimization studies in both children and pregnant women should be counted as a means of forestalling drug resistance and preserving drug efficacy [122, 123]. The most recent example of this is in PPQ, shown to be suboptimally dosed in the youngest age

groups, since rectified with newer dosing schedules for DHA-PPQ products that nearly double the conventional dose for the smallest children [124]. Other immediately available means of combatting drug resistance are efforts to curtail the circulation of substandard, falsified, and obsolete (e.g., artemisinin or pyrimethamine monotherapy) antimalarial drugs [125–127].

Transmission-blocking interventions are essential to curbing the spread of drug-resistant parasites, some strains of which have shown greater transmissibility than wild-type parasites [128, 129]. Chemotherapeutically, this entails the use of agents with activity against gametocytes, the transmissible stage of the parasite. The only true *P. falciparum* gametocytocides currently available are the 8-aminoquinolines, primaquine, and tafenoquine. Different ACTs appear to have differing anti-gametocyte efficacies, but fail to clear gametocytes to the extent of 8-aminoquinolines [130, 131]. The threat of drug resistance should motivate the global community to more ardently promote wider adoption by national malaria control programs of single low-dose (SLD) primaquine as recommended, in 2012, by the WHO for areas of low transmission and areas of artemisinin resistance [132–134]. This will require further demonstration of SLD primaquine's safety in areas with prevalent glucose-6-phosphate dehydrogenase (G6PD) deficiency, associated with drug-induced hemolytic anemia, as recently done in South Africa, Tanzania, and Central America [135–137], and would benefit from affordable point-of-care tests of G6PD activity.

The WHO endorses three strategies for chemopreventive or presumptive treatment in high-risk groups: intermittent preventive treatment in infants (IPTi), intermittent preventive treatment in pregnancy (IPTp), and seasonal malaria chemoprevention (SMC) [138–140]. IPTi and IPTp are recommended in areas of moderate to high *P. falciparum* transmission, and the IPTi guidelines limit its use to areas where the frequency of the dihydropteroate synthase (*dhps*) K540E mutation is ≤ 50% [140]. SMC is recommended in areas of high seasonal transmission and is currently carried out in the Sahel region of Africa [141]. Thirty-nine million children live in areas where SMC is recommended, and modeling suggests that SMC, if widely implemented, could avoid over 21 million malaria cases and 95 thousand deaths annually [142].

Current guidelines for the three preventive strategies propose SP or SP in combination with amodiaquine (AQ) [138–140]. Declines in efficacy have occurred in connection with increasing prevalence of mutations in the antifolate resistance genes *dhps* and dihydrofolate reductase (*dhfr*) [143••, 144••]. Alternatives to SP and AQ-SP have therefore been examined—including ACTs, CQ combined with azithromycin, and others [145•, 146, 147•, 148•, 149–152]—with a particular focus on PPQ-containing regimens given its relatively long half-life, but the readiness with which PPQ resistance may arise warrants caution. All told, IPTi, IPTp, and SMC programs should follow a coherent antimalarial policy that adheres to the principles of anti-infective stewardship and relies on up-to-date drug resistance surveillance data, avoiding first-line agents to minimize drug pressure and thereby helping preserve their efficacy in clinical use.

Lastly, the history of mass drug administration (MDA) for malaria and controversies around its role in malaria control—and in promoting drug resistance—extend back almost 100 years and remain a topic for debate [153]. The bulk of the evidence indicates against its utility except under very constrained circumstances, reflected in current WHO recommendations which limit MDA to complex emergency situations and to areas on the verge of malaria elimination with robust malaria control measures already in place and minimal risk of reimportation [153, 154].

Drug Discovery and Development to Counter Antimalarial Resistance

Countering antimalarial drug resistance requires new effective therapies. An active antimalarial drug development pipeline has been fostered through private-public partnerships, open-source access to compound libraries, and advances in high-throughput screening that includes the ability to assess stage specificity of candidate compounds [155, 156••, 157]. Groundbreaking work in parasite genomics and transcriptomics has accelerated the identification of potential drug targets [158••, 159]. Antimalarial drug development has also harnessed the latest in clinical pharmacology science, including phase 0 microdose studies, novel nanoformulations of antimalarial compounds, the development of antimalarial biologics, and both in vitro and in silico pharmacometric simulations [160–162]. Updated pharmacodynamic models of parasite clearance and antimalarial activity supplement conventional approaches to how drug efficacy is assessed, helping to inform rational drug development and posology [163–165]. Growing capacity in sub-Saharan Africa for locally conducted phase 1 and 2, studies, which included the first-in-human study of a novel *Plasmodium* phosphatidylinositol 4-kinase (PI4K) inhibitor, lends promise that early drug development can increasingly take place in clinically relevant populations [116, 166]. These efforts have led to a robust pipeline of next-generation and novel classes of antimalarials with activity against artemisinin-resistant *Plasmodia* that includes the synthetic ozonides, inhibitors of PI4K, and monoclonal antibodies [166–168, 169••, 170–172].

Advances in Molecular Surveillance and Bioinformatics

Molecular surveillance relies on *Plasmodium* genomics for the identification of drug resistance markers. The use of genome-wide association studies (GWAS) and other population genetic tools to characterize the emergence, mechanisms, and movement of drug-resistant parasites has revolutionized our understanding of the molecular epidemiology of antimalarial resistance with implications for the Global Technical Strategy and national malarial control programs [173].

Next-generation sequencing (NGS) methods, such as targeted amplicon deep sequencing (TADS), are increasingly used to monitor molecular markers of resistance [174–178]. By enabling high-sensitivity, high-throughput analysis of molecular markers, the declining costs and increasing data yield of NGS have positioned it to replace traditional PCR-based methods [179]. Pooled sequencing of individual samples from infected individuals using NGS substantially boosts the number and rate of sample analysis [180–182]. Keeping apace of these innovations in sequencing technology are innovations in bioinformatic methods for the analysis of TADS data [174, 183–185].

At present, several hurdles remain for the widespread implementation of NGS-based methods of molecular surveillance of antimalarial drug resistance. These challenges have recently been reviewed by Ishengoma et al. [186]. The requirement for advanced infrastructure and training amplifies these challenges in the resource-deprived settings where malaria is endemic.

Translational Genomics for Malaria Control

Like in other areas of antimicrobial drug resistance research, GWAS has become an essential tool for the identification of genetic regions and loci related to antimalarial resistance. Recently reviewed elsewhere by Volkman et al. [187], GWAS has been used to confirm previously suspected drug resistance polymorphisms and identify mechanisms of resistance to new classes of antimalarials [109, 188–195]. Because the emergence of resistance leaves specific signatures in the parasite genome, GWAS and other population linkage approaches can provide clues to how antimalarial resistance emerges and spreads [196–202]. This provides practicable knowledge that can inform surveillance for, anticipatory strategies against, and containment of drug resistance.

For example, with the identification of polymorphisms in *pfk13*, population linkage studies provided evidence that the mutations associated with artemisinin resistance emerged independently multiple times throughout the Greater Mekong Subregion of Southeast Asia, alerting programs to the fact that containment alone will be insufficient [193]. Subsequent studies showed that successful artemisinin-resistant lineages spread across the region outcompeting other parasites [203–205]. Genomic data can be translated into actionable information for malaria control through use of different visualization tools such as landscape genetics. For artemisinin resistance in the Greater Mekong Subregion, the use of estimated effective migration surfaces has been proposed to demarcate barriers and corridors for parasite migration [206, 207•]. These kinds of approaches have the potential to identify key locations at national and regional levels where interventions should be prioritized for halting the spread of resistance.

While genomic-based approaches have predominantly been used in areas of low transmission where infections tend to be monoclonal, advances in bioinformatics have allowed for similar studies to be undertaken in high-transmission regions in sub-Saharan Africa where infections with multiple strains are common. New deconvolution methods for genomic data allow for more robust interpretations of genomes in polyclonal samples [208, 209]. These early methods have already been used to trace parasite migration between Tanzania and the Zanzibar archipelago [210]. Another approach has leveraged molecular inversion probes to describe long-range migration of parasites and temporal changes in antimalarial resistance in the Democratic Republic of the Congo [115•, 211••, 212]. Methods for estimating the propagation of resistance in these high-transmission settings require further development before being routinely deployed.

The continual surveillance of parasite population by genomics has the potential to help identify new mechanisms of resistance early in the course of drug deployment and formulary changes. In combination with other genomics-based approaches, this could hasten the recognition of concerning signals for antimalarial resistance in parasite populations. Recent studies using a combination of in vitro resistance evolution and whole genome sequencing (IVIEWGA) have started to map the *P. falciparum* "resistome" [213••, 214]. This approach identified new potential resistance mechanisms and possible drug targets. Several of the putative genes associated with resistance have now been shown to have signals of selection in population genomics studies [190, 211••]. Meanwhile, the identification of novel mechanisms of resistance and new artemisinin-resistance candidates is under investigation using IVIEWGA [215–217]. There is an ongoing need to identify alternative resistance mechanisms in sub-Saharan Africa where clinical signs of partial resistance (prolonged parasite clearance) have been described without evidence of *pfk13* mutations [60•, 218].

Advances in gene editing have also proven pivotal. The ability to deliver single point mutations and gene deletions with modern gene editing platforms, such as zinc finger endonuclease and CRISPR/Cas9, has accelerated and expanded genetic studies of antimalarial resistance [219–224]. Gene editing was key to understanding the functional impacts of

individual mutations in *pfk13*, a significant achievement given the complex genetic backdrop of the gene which has a high degree of underlying variations with little to no functional roles [61, 110, 225]. Functional genomics has also been used to validate resistance mutations for other antimalarials including CQ, PPQ, ozonides, and piperazine compounds [226–229, 230••, 231]. While much of this work has been in *P. falciparum*, gene-editing technologies are also being applied to other human malaria parasites [232]. Current approaches, however, are limited by low efficiency and high numbers of unexpected recombination events. Gene editing in *Plasmodium* spp. is uniquely challenging due to the parasites' AT-rich genome, lack of canonical DNA repair pathways, absence of nuclear genomic RNAi, and attrition of transfecting plasmids during cell division [233–235]. Over the last couple of years, innovations in CRISPR/Cas9-based tools in *P. falciparum* and other species have begun to overcome these challenges [236•, 237].

Conclusion

Centuries before Paul Erlich ushered in the era of antimicrobial chemotherapy, malaria remedies were gotten by our ancestors from *Cinchona* and *Artemisia* plant species, original sources of what remain the two most widely used antimalarial pharmacophores. Signs of antimalarial drug resistance were first evident over 100 years ago, and a punctuated march of drug resistance across the globe proceeded from almost every new introduction of antimalarials in the years that followed [19, 31]. Today, we have increasingly sophisticated tools for surveilling, containing, and combatting the emergence, spread, and harms of antimalarial drug resistance. Yet malaria control is backsliding. The contravening forces of political instability, donor fatigue, climate change, and, recently, competing public health priorities and economic recession caused by the coronavirus pandemic are complicit. The expansion of drug resistance to ACTs poses at least an equal threat. Updates in drug development, clinical pharmacology, parasite genomics, transcriptomics, and bioinformatics, with their efficient and judicious application, can reclaim lost ground in the WHO Global Technical Strategy. A forward-looking agenda that helps preserve inasmuch as possible the efficacy of current agents through increased antimalarial stewardship, speeds the arrival of new agents and treatment regimens, and fully exploits nimble and scalable genomics-based platforms for discovery and surveillance can replace vulnerability with opportunity.

Declarations

Conflict of Interest The authors declare no competing interests.

Human and Animal Rights and Informed Consent This article does not contain any studies with human or animal subjects performed by any of the authors.

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