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New Challenges in Malaria Elimination

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Abstract

In recent years, efforts to eliminate malaria has gained a tremendous momentum, and many countries have achieved this goal — but it has faced many challenges. Recent COVID-19 pandemic has compounded the challenges due to cessation of many on-field operations. Accordingly, the World Health Organization (WHO) has advocated to all malaria-endemic countries to continue the malaria elimination operations following the renewed protocols. The recent reports of artemisinin resistance in *Plasmodium falciparum* followed by indication of chloroquine resistance in *P. vivax*, and reduced susceptibility of synthetic pyrethroids used in long lasting insecticide nets are some issues hindering the elimination efforts. Moreover, long distance night migration of vector mosquitoes in sub-Saharan Africa and invasion of Asian vector *Anopheles stephensi* in many countries including Africa and Southeast Asia have added to the problems. In addition, deletion of histidine rich protein 2 and 3 (*Pfhrp2/3*) genes in *P. falciparum* in many countries has opened new vistas to be addressed for point-of-care diagnosis of this parasite. It is needed to revisit the strategies adopted by those countries have made malaria elimination possible even in difficult situations. Strengthening surveillance and larval source management are the main strategies for successful elimination of malaria. New technologies like Aptamar, and artificial intelligence and machine learning would prove very useful in addressing many ongoing issues related to malaria elimination.

Keywords: Malaria, *Plasmodium vivax*, *P. falciparum*, drug resistance, vector invasion, night migration, insecticide resistance, gene deletions, surveillance, larval source management, elimination, Aptamar, Artificial intelligence, machine learning, COVID-19

1. Introduction

In the past two decades, tremendous progress has been made in the fight against malaria. A great deal of new knowledge on malaria parasite [1], insights in vector biology and control have helped target interventions resulting in substantial transmission reduction globally [2–4]. In 2019, World Health Organization (WHO) estimated 229 million malaria cases and 409,000 deaths in 87 malaria-endemic countries with large concentration of the total malaria burden (94%) in Africa [5]. Global malaria cases declined by 27% between 2000 and 2015, and only 2% between 2015 and 2019 indicating the slow progress rate in this period (**Figure 1**) [5]. Of the 29 countries that contributed 95% of the global malaria cases, Nigeria alone accounted for the highest at 27% followed by Democratic Republic of the Congo (12%), Uganda (5%), Mozambique (4%) and Niger (3%). A compiled data of global malaria cases

Figure 1. Trends of global malaria 2000 to 2019. Nigeria alone continue to contribute majority of the cases; in 2019 contributed 27% of total global malaria cases. Compiled from WHO data.

from 2000 to 2019 showed a declining trend (**Figure 1**). But the trend between 2015 and 2019 is not a good indication of malaria elimination goal from 2016 to 2030, as envisaged by the Global Technical Strategy (GTS) of WHO. The population at risk living in the WHO African Region increased from about 665 million in 2000 to 1.1 billion in 2019. The WHO South-East Asia Region (SEAR) accounted for about 3% of the burden of malaria cases globally. India is the major contributor in this region sharing 2% of the total global malaria cases. On the other hand, seven countries namely Algeria, Kyrgyzstan, Uzbekistan, Argentina, Paraguay, Maldives and Sri Lanka have been certified malaria free by WHO from 2015 to 2019 [6]. Among the E-2020 countries: China, Iran, Timor-Leste, Malaysia and El-Salvador reported zero malaria in 2018 setting a precedent that malaria elimination is possible by strengthening surveillance system (**Figure 2**) [7]. In 2020, the whole world experienced an unprecedented situation of COVID-19 pandemic threatening malaria elimination efforts. Most of the public health services were diverted towards managing and containing this severe form of infection caused by SARS-COV-2 virus. Aims and aspirations are high for living in the malaria-free world, yet there are multiple challenges for realizing the goal of malaria elimination by 2030. Enumerated below are some of

Figure 2. Global malaria burden. Countries with indigenous cases in 2000 and their status by 2019. Source WHO [5].

issues which should be addressed to strengthen the health systems for the achieving coveted goal of malaria elimination in due time.

2. Search methods

Searched MEDLINE (PubMed); CABS Abstracts; checked the reference lists of all studies identified by the search. Also performed Google Search on specific topics. Examined references listed in review articles and previously compiled bibliographies.

3. The challenges

Besides the current impending threat of COVID-19, many more challenges are being faced in defeating malaria. Some of these are: (a) deletions of *PfHPR2/3* genes in *Plasmodium falciparum* at the point-of-care diagnosis, (b) drug resistance to parasites, (c) migration of parasite strains to newer areas, (d) migration of drug-resistant parasites in low-transmission settings, (e) multi-insecticide resistance in vector mosquitoes, (f) poor disease surveillance, (g) invasion of *Anopheles stephensi* in Africa and elsewhere, (h) long-distance migration of vector mosquitoes in sub-Saharan Africa, and (i) unmet funding drift.

3.1 Gene deletions compromising performance of Rapid Diagnostic Test (RDT) Kits

Rapid diagnostic tests (RDTs) detect species-specific antigens of *P. falciparum* and *P. vivax* parasites present in the blood of infected patients. Histidine rich protein 2 and 3 (*Pfhrp2*, *Pfhrp3*) are widely used in RDTs for point-of-care diagnosis of *P. falciparum*. Naturally occurring deletions of these genes are emerging threat to malaria detection and treatment, management and elimination. *Pfhrp2/3* deletions are increasingly reported from all malaria-endemic regions. Deletion of *Pfhrp2/3* genes in *P. falciparum* is one of the major issues for diagnosis of this dominant species globally. A recent detailed global review by WHO clearly showed a huge problem exists in Amazon basin and Eritrea [8, 9].

In India, two major studies reported the problem is limited to 0 to 23% [10, 11]. The global distribution and prevalence of these deficient genes is presented in **Figures 3** and **4**. In the Peruvian Amazon, there was emergence of a drug-resistance profile BV1 clonal lineage that was distinctly different from the previous genotype found in the region [12]. The BV1 lineage profile posed a significant problem because the strain is multidrug-resistant and escapes detection by *Pfhrp2*-based RDTs secondary to *Pfhrp2/3* deletions. The hypothesis was that the BV1 strain had emerged as a successful parasite lineage for transmission by different vectors and had contributed to the increased malaria burden recently observed in some Amazonian regions [13].

As per the report of WHO, of the 39 reports published in 39 countries, 32 (82%) reported *Pfhrp2* deletions. However, deletions are still unclear when variable methods in sample selection and laboratory analysis are performed. From the 16 published documents in 15 countries between 2019 and September 2020, *Pfhrp2/3* deletions were confirmed in 11 countries from 12 reports. These countries are China, Equatorial Guinea, Ethiopia, Ghana, Myanmar, Nigeria, Sudan, Uganda, United Kingdom (imported malaria cases from various malaria endemic countries), the United Republic of Tanzania and Zambia. However, no deletions were detected in France (among returning travelers), Haiti, Kenya and Mozambique [5].

Figure 3.
Highest percentage of *Pfhrp2* deletions in *P. falciparum* cases tested. Source: WHO [8] and Thompson et al. [9].

Figure 4.
Weighted average estimates for *Pfhrp2* deletions in *P. falciparum* patients tested by country. Source: WHO [8] and Thompson et al. [9].

3.2 Drug resistance

The WHO recognises that drug resistance is one of the main concerns which requires periodic monitoring and appropriate drug policy in place to stay a step ahead arresting development and spread of drug-resistant malaria. In recent years, molecular monitoring and surveillance of mutant markers has gained pace to help the programme significantly providing an early indication of possible drug failure helping institutes alternated therapeutic regimen for radical cure [14]. *P. falciparum* and *P. vivax* are the two common human malaria parasite species, of which the former is widespread and continually evolving to be drug-resistant (**Figure 5**).

3.2.1 *Plasmodium falciparum*

Among all human malaria parasite species, *P. falciparum* is the most dominant species especially in sub-Saharan Africa. Recent first high-resolution global map of

Figure 5.
*History of Chloroquine-resistant *P. falciparum* malaria. Origin of resistance in 1957 from South East Asia and global spread in subsequent years. Source: Packard [16].*

falciparum mortality, prevalence and incidence illustrated a rapid decline in burden between 2005 and 2017. However, 90.1% of people continue to reside within sub-Saharan Africa which accounted for 79.4% of cases and 87.6% of deaths in 2017 [15].

Besides *in-vivo* follow-up studies for monitoring therapeutic efficacy, the Technical Consultation Committee of WHO recommended the use of malaria molecular surveillance (MMS) for implementation in malaria elimination and control. Monitoring of molecular marker and therapeutic efficacy studies help to identify and track the prevalence of molecular mutations associated with drug resistance.

3.2.1.1 Chloroquine resistance markers

Once Chloroquine was a first-line drug for malaria treatment but has become obsolete for treatment of *P. falciparum*. Chloroquine-resistant *falciparum* malaria first reported in Thailand in 1957 (**Figure 5**). Subsequently, it spread through South and Southeast Asia and by the 1970s in sub-Saharan Africa and South America [16]. In India, predominant SVMNT haplotype of *PfCRT* K76T mutation was first reported in 2004 [17]. Presence of SVMNT haplotype rather than CVIET specific of the African/Southeast Asian haplotype in India was suggestive of prevalence of chloroquine resistance in Indian strains of *P. falciparum* [17]. This helped replacing chloroquine as first-line drug for *P. falciparum* malaria to artemisinin-based combination therapy (ACT). But a study in 2020 in Mizoram (bordering Myanmar), reported prevalence of CVIET haplotype indicating its presence in this region of India [18]. Molecular surveillance of markers has helped trace the route of migration of drug-resistant malaria in the world (**Figure 5**).

3.2.1.2 Sulfadoxine-Pyrimethamine (SP) resistance markers

Dihydropteroate synthase (*dhps*) inhibitors, such as sulfadoxine, and dihydrofolate reductase (*dhfr*) inhibitors, such as pyrimethamine, disrupt parasite's folate synthesis. Antifolate resistance has been associated with point mutations in the *pf dhps* and *pf dhfr* genes. Point mutations at codons 16, 51, 59, 108 and 164 of *pf dhfr* inhibit its activity, and the parasite becomes resistant to pyrimethamine. Two mutations C59R and S108N in *pf dhfr* were recorded to be prevalent in India. While mutations at 436, 437, 540, 580 and 613 of *pf dhps* reduce the substrate binding capacity and confer resistance to sulfadoxine. Three mutations S436A, A437G and K540E were found associated with *pf dhps* [19].

Intermittent preventive treatment in pregnant women (IPTp) and in infants (IPTi) sulfadoxine-pyrimethamine (SP) is recommended to prevent *P. falciparum* malaria in moderate to high transmission areas of sub-Saharan Africa. But the major problem lies on resistance of SP to *P. falciparum*. As per WHO protocol, SP is no more effective for IPTp and IPTi in most of eastern Africa and parts of central Africa. WHO recommends countries to withdraw IPTp when the prevalence of *pfdhps* 540E is >95% and *pfdhps* 581G >10%, and IPTi when the prevalence of *pfdhps* 540E is >50% [20]. However, a recent meta-analysis found that IPTp still reduces the risk of low birth weight even in areas where high level of *pfdhps* and *pfdhfr* quintuple mutant haplotypes are present. But in areas where the sextuple mutant parasite harbouring the additional *pfdhps* 581G mutation IPTi appears to have no significant protective effect. Therapeutic alternatives to SP-IPTp are needed in areas where the prevalence of the sextuple mutant parasite exceeds 37% [21].

3.2.1.3 Artemisinin resistance marker

Artemisinin-based combination therapy (ACT) is presently the drug of choice for treatment of resistant *P. falciparum* malaria. Mutation at *Pfkelch13* propeller gene (*K13*) responsible for its role in Artemisinin resistance supposedly originated in Southeast Asia [22]. WHO has prepared a list of validated *PfKelch13* mutations of partial resistance to artemisinin. These are F446I, N458Y, M476I, Y493H, R539T, I543T, P553L, R561H, P574L and C580Y, and also the candidate markers P441L, G449A, C469F/Y, A481V, R515K, P527H, N537I/D, G538V, V568G, R622I and A675V [5]. A recent review in the *NEJM*, a worldwide map of *K13*-propeller polymorphism found no evidence of Artemisinin resistance outside Southeast Asia and China, where resistance-associated *Pfkelch13* mutations were confined [23]. Later, after comparing the *Pfkelch13* R561H genome sequence to other samples taken from all over Africa, and sequences taken from South America and Bangladesh, it was observed that the artemisinin-resistant strain of the parasite is tightly clustered with Rwandan parasites indicating artemisinin resistance in Africa [24].

In 2019, a report from eastern India indicated the presence of two mutations G625R and R539T in 5/72 *P. falciparum* cases treated with artemisinin that linked to its presence of resistance [25]. But C580Y in *kelch13* in Southeast Asia and Mekong Delta areas were predominant [26]. Following high rates of Artesunate (AS) + Sulfadoxine-Pyrimethamine (SP) treatment failure in the north-eastern provinces in 2013, India changed its treatment policy in those provinces to Artesunate-Lumefantrine (AL); while AS+SP remains effective elsewhere in the country [5]. Moreover, a specific lineage of plasmepsin amplification (*PLA1*) has been detected that caused dihydroartemunate-piperquine (DHA-PPQ) treatment failure in western Cambodia due to its use as mass drug administration campaign [5]. Both the lineages of *k13* (*KEL1*) and *PLA1* have compounded the problem of DHA-PPQ resistance in this region [13]. In Guyana, between 2010 and 2017, the C580Y mutation also emerged independently. However, recent studies indicated 100% of samples were found to be wild type, indicating that the mutation may be disappearing in Guyana. There is no indication of lumefantrine failure in Africa [5].

3.2.2 *Plasmodium vivax*

P. vivax was believed to be a benign malaria, but this phenomenon has changed in recent years for its appreciable causes of morbidity and mortality [27]. In recent years, this species has amounted to cause a significant public health burden [28, 29]. Even though there has been a considerable decrease on the burden, still over four billion people are living at risk of this infection. In 2017, *vivax* transmission was

reported from 49 countries across Central and South America, the Horn of Africa, Asia, and the Pacific islands. *P. vivax* is the predominant species in almost two-thirds of co-endemic countries. Recent estimates, incorporating national surveillance data, prevalence surveys, and geospatial mapping, have revised the global burden to between 13.7 and 15 million cases in 2017 [30, 31]. It is assumed that ACT is widely used across Africa to combat falciparum malaria that may be favouring adaptive selection for *P. vivax* for its failure. Additionally, a better understanding is needed on the mechanism of erythrocytes invasion in Duffy-negative individuals who were previously thought to be protected against *P. vivax* malaria [32]. An estimated 82% (11.7 million cases) of the global vivax burden comes from four high-burden countries, i.e. India, Pakistan, Ethiopia, and Sudan [33].

In most countries, chloroquine is still being used for the treatment of blood stages of *P. vivax* malaria. However, chloroquine-resistant (CQR) *P. vivax* has emerged in many areas with variable degree of clinical efficacy [34]. High-grade chloroquine resistance is reported from the intense transmission area of the island of Papua (Indonesia and Papua New Guinea), and Sabah (Malaysia) where malaria elimination is well within reach [33]. To combat the declining susceptibility of *P. vivax* to chloroquine, five countries that include Indonesia, Papua New Guinea, Solomon Islands, Vanuatu and Cambodia have adopted a policy of universal ACT for both *P. falciparum* and *P. vivax* [33]. In other areas where CQR is low-grade and transient, chloroquine remains the main drug of choice for treatment of *P. vivax* malaria including four high-burden countries [33, 34].

Molecular monitoring of chloroquine resistance in *P. vivax* is an integral part of national malaria control programme. The *P. vivax* ortholog of *pfcr*, referred as *pvcrt-o*, was characterized nearly two decades ago [35]. A lysine (AAG) insertion at amino acid 765 position 10 (K10) was discovered in Southeast Asian strains and suggested to be associated with chloroquine resistance where high dose of chloroquine is recommended [36].

In India, chloroquine is still effective for treating *P. vivax* malaria. Almost half of the malaria cases are due to *P. vivax* mostly present in low transmission settings [28]. A study in 2013 in Kolkata by Ganguly et al. observed non-synonymous polymorphism in *pvcrt-o* and *pvm-dr1* and concluded no indication of chloroquine resistance in *in vivo* study [37]. But the report in 2018 from Mangalore indicated the presence of chloroquine resistance involving two genes in K10 insertion in *pvcrt-o* and F1076L mutations in *pvm-dr1* [38]. Subsequently, such mutants were also detected in Puducherry, Mangaluru (Mangalore), Cuttack and Jodhpur [39]. A study in Chandigarh, North India reported double mutations in K10 insertions at 17.5% and 9.5%, in complicated and uncomplicated *P. vivax* groups, respectively. *Pvm-dr1* gene analysis revealed 100% double mutants (T958M/F1076L) in the complicated, and 98.7% in the uncomplicated group, respectively. Presence of a single triple mutant T958M, F1076L and Y1028C was observed in the uncomplicated group [40]. More number of such cases were reported from Peru in amino acid changes at positions 976F and 1076 L for *pvm-dr1* [41], Afghanistan [42], Malaysia [43], Australia (among travellers) [44] and Brazilian Amazon [45].

Tackling *P. vivax* malaria is a herculean task due to its complex biology of hypnozoites that cause relapse. Primaquine – an 8 aminoquinoline is widely used for radical cure of malaria. But its use has been restricted in infants, pregnant women and also in G6PD-deficient individuals. WHO recommends to administer primaquine for radical cure of vivax relapse. More than 180 different G6PD deficiency alleles reported [5]. Cytochrome P450 2D6 is an enzyme that in humans is encoded by the CYP2D6 gene. CYP2D6 is primarily expressed in the liver, and is associated with primaquine tolerance [46]. So, both G6PD deficiency and CYP2D6 are contraindications to primaquine administration. In certain situations, high-dose

primaquine regiments are currently recommended for radical cure of vivax malaria in Southeast Asia and Oceania [47].

Mutations at the *pvdhps* and *pvdhfr* loci leading to antifolate resistance are commonly found in *P. vivax* isolates from *P. vivax*-infested areas. DHFR mutations in *Plasmodium vivax* in Indonesia failed to therapeutic response to SP [48]. Triple mutations in *pvdhfr* was reported in Mangalore, India [49]. Thus, antifolates are not currently recommended as a first-line treatment of vivax malaria [50].

4. Invasiveness of *Anopheles stephensi*

Anopheles stephensi was originally described by Liston in 1901 from a village Ellichpur (now Achalpur) in Amravati district, Maharashtra state of India. This species is a principal malaria vector in urban India, and is considered imminent threat to malaria elimination efforts [51]. This species has three variants i.e. type, intermediate and *mysorensis* based on its egg morphometric analysis. Both type and intermediate forms are efficient vectors in rural and urban settings, but type form is the main and very efficient vector in urban settings, whereas variety *mysorensis* is considered as a rural vector with limited role. Laboratory studies have shown that all the three variants are capable of harbouring rodent malaria *P. berghei* [52] and *P. yoelii nigeriensis* [53] parasites respectively. *An. stephensi* is an efficient vector for transmitting *P. falciparum* and *P. vivax* malaria equally in the field [54].

Recent reports indicate that *An. stephensi* is expanding its geographical range crossing from the Arabian Gulf into the Horn of Africa where it has been reported in Djibouti City in 2012 [55], in Ethiopia in 2016 [56] and the Republic of Sudan [57]. In 2016, type form of this species was found for the first time in Sri Lanka [58]. Emergence of *An. stephensi* has been associated epidemiologically with an unusual resurgence in local malaria cases in Djibouti city [55]. This species is basically a container breeder and sympatric co-share breeding with *Aedes aegypti* [59]. In Sri Lanka, *An. stephensi* has been found to breed in salt water [58]; while it breeds in clear water in central regions of urban setting of Africa, but with all probability it

Figure 6. The new “out of range” occurrence of *An. stephensi* in the Arabian Peninsula and Horn of Africa showing the 358 *An. stephensi* site locations used in final species distribution models (SDMs) colour coded by the decade in which they were sampled. Source: Sinka et al. [61] with permission.

may even breed in polluted water in Africa where most malaria vectors breed in such waters [60]. Sinka et al. prepared evidence-based maps predicting the possible locations of *An. stephensi* across Africa where it could establish if its spread is unchecked. The high probability maps predict the presence of *An. stephensi* in many urban cities across Africa where an estimated over 126 million people live (**Figure 6**) [61].

5. Long-distance travel of mosquitoes

Transportation of mosquitoes from one place to other through surface transportation or air route is well known. The accidental invasion of *An. gambiae* in 1930 in Natal, Northeast Brazil from Dakar, Senegal most likely adult mosquitoes that travelled in planes or ships, as no larvae were found is one such example [62]. Generally, mosquitoes can fly within a few kilometers from their breeding habitats. But long-distance migration across hundreds of kilometers during night hours is very revealing, and have implications in malaria eradication efforts. Recent study in Sahel desert of Mali in Africa, Huestis et al. reported that mosquitoes could possibly migrate up to 300 kilometers for 9-hour flight duration. Sticky nets tethered to helium-filled balloons fixed in the study villages suspended at set altitudes ranging from 40 to 290 metres above mean sea level were launched at about 10 consecutive nights each month over a span of 22–32 months. Ten species, including the primary malaria vector *An. coluzzii*, were identified among 235 Anopheline mosquitoes that were captured during 617 nocturnal aerial collections. Annually, the estimated number of mosquitoes that possibly could have migrated at altitude that cross a 100-km line perpendicular to the prevailing wind direction included 81,000 *Anopheles gambiae* ss, 6 million *An. coluzzii* and 44 million *An. squamosus*. Females accounted for more than 80% of all of the mosquitoes, and 90% of them had taken a blood meal before their migration, and studies suggest mosquito infection rates in the region are between 0.1% and 5% [63, 64]. Annually, the estimated numbers of mosquitoes at altitude that cross a 100-km line perpendicular to the prevailing wind direction. The authors concluded that millions of malaria vectors that have previously fed on blood may migrate frequently over hundreds of kilometers, and spread malaria [64]. Thus the successful elimination of malaria depends on how the sources of migrant vectors can be identified and controlled.

6. Insecticide resistance

Resistance to insecticides in vector mosquitoes is an organic *de novo* biological process. This has caused a major setback in achieving malaria elimination. Recently an update at global scale on insecticide resistance in malaria vectors has been enumerated [65]. WHO documented a cumulative total of 82 countries reported data on insecticide resistance from 2010 through 2019. Resistance of malaria vectors to insecticides threatens malaria control and elimination efforts. Commonly used insecticides are synthetic pyrethroids, organophosphates, carbamates and the rarely used organochlorine dichlorodiphenyltrichloroethane (DDT) [5].

Sources of insecticides in the environment include the application of insecticide-based vector control interventions for public health such as Indoor Residual Spray (IRS) and the application of agricultural insecticides, which include the same class of insecticide as those used in vector control programme [66]. Also pesticide contamination in water bodies is also a cause of selection pressure for resistance in mosquito larvae [67]. In response to the Roll back malaria (RBM) initiative, long lasting insecticide nets (LLIN) coverage increased markedly across Africa from 2005 [68], while

IRS usage has been restricted in smaller areas [69]. Either permethrin or deltamethrin was used initially in insecticide-treated nets (ITNs), and now α -cypermethrin is most commonly used in LLINs. Deltamethrin, λ -cyhalothrin, and DDT have been used for IRS for over 20 years. In 2003, first α -cypermethrin was used in mass campaigns. Deltamethrin has been the sole pyrethroid along with DDT and other non-pyrethroid insecticides are used in IRS from 2015 [69]. There are conflicting reports on the cause of insecticide resistance in field mosquitoes following the introduction of LLINs, IRS or both. Some studies documented an increase in resistance [70]; whereas others observed no such evidence after implementation of these intervention strategies [71].

Anopheles culicifacies is the main vector out of six primary malaria vectors and responsible for 2/3 malaria cases distributed across rural India. An tempo-spatial analysis of insecticide susceptibility status between 1991 and 2016 from 145 districts in 21 states indicated resistance to at least one insecticide in 70% (101/145) of the districts – mostly to DDT and malathion whereas, its resistant status against deltamethrin varied across the districts [72]. Similar trend was also reported in Odisha – a highly malaria-endemic state in India [73].

In India, National Vector Borne Disease Control Programme (NVBDCP) has distributed about 50 million LLINs to malaria-endemic communities for intervention during 2016–2018, and to 126 million population at risk [74]. The lower efficacy of synthetic pyrethroids to vector mosquitoes is a matter of concern. Certain major drawbacks of LLINs include feelings of suffocation in humid tropical climate, and some traditional practices compelling the users to wash the nets more frequently than prescribed protocol. Another risk of using LLINs is host switching and possible horizontal transmission potential in the endemic areas [73].

Resistance of malaria vectors to pyrethroids may pose a serious problem in achieving the malaria elimination goal. The six countries Cambodia, China (Yunnan Province), the Lao People’s Democratic Republic (PDR), Myanmar, Thailand and Viet Nam of the Greater Mekong Subregion (GMS) have made significant gains in their battle to eliminate malaria by 2030. In recent years, there has been a remarkable progress towards elimination of the disease. Between 2012 and 2018, the reported number of malaria cases fell by 74%; malaria deaths fell by 95% over the same period. However, Cambodia contributes almost half of malaria in this zone of which 85% are *P. vivax*. This may be due to high degree resistance of pyrethroids [75] (Figure 7).

Figure 7. Resistance of malaria vectors to pyrethroids in the Greater Mekong Subregion, 2010–2019. Source: WHO [75].

Figure 8.
Comparative genomics of vector mosquitoes to understand difference in vectorial capacity. Source WHO [13].

Another important aspect is to understand and track gene flow in Anopheline mosquitoes. This is complex given the amount of genetic diversity that exists within mosquito populations. **Figure 8** depicts a clear picture how comparative genomics can be applied to understand differences in vectorial capacity and their impact on malaria transmission [13].

7. Poor disease surveillance

Disease surveillance is the key intervention strategy to support malaria elimination. A new guideline promulgated by WHO in 2018 reinforced the GTS and framework for elimination principle that identified surveillance as the main elimination strategy. But poor surveillance continues to derail the elimination efforts. Strengthening surveillance system in many countries eliminated malaria successfully. Aiming malaria elimination, a standardized surveillance system landscaping compared with ideal system were conducted in 16 countries aiming malaria elimination. Assessment was done in 2015 and 2016 across the Greater Mekong Subregion (Cambodia, Laos, Myanmar, and Vietnam), Southern Africa (Botswana, Mozambique, Namibia, South Africa, Swaziland, and Zimbabwe), Hispaniola (Dominican Republic and Haiti), and Central America (Costa Rica, Guatemala, Honduras, and Panama). This landscaping analysis provided a clear framework that identified multiple gaps in current malaria surveillance systems. It is important to close these gaps identified which will allow countries to deploy resources more efficiently, track progress, and accelerate towards malaria elimination [75]. Rapid reporting and information on geolocation have been the strength of malaria control system in Zanzibar for over a decade resulting in low transmission of malarial cases [76]. However, falciparum malaria remains a problem in Zanzibar and Swaziland [77–79]. China adopted and continues the ‘1-3-7’ surveillance strategy, whereby case notification occurs within one day, case investigation within three days and foci investigation and targeted action within seven days. To avoid transmission and re-establishment, monthly bulletins are issued on reported and detected cases. Training, technical support and supervision are provided regularly to sustain capacity [80]. In Mangalore, India a ‘1-3-7-14’ strategy is under operation using digital TAB-based smart surveillance which focuses on real time micromanagement of each malaria positive case and vector control operation [81, 82]. Digital surveillance

and bridging the surveillance gaps are two major issues have been advocated to accelerate towards malaria elimination [83].

8. Discussion

In the last five years the GTS milestone has drawn a detailed road-map to eliminate malaria from 2016 to 2030. It can be seen from the **Table 1** that all the 58 countries in the Europe and Central Asia territories, and in other territories around 50% achieved malaria elimination except Sub-Saharan Africa. This means the real problem still exists in this territories [83]. This also reflects in the world malaria report. In November 2018, WHO together with RBM Partnership to End Malaria has launched the ‘high burden to high impact’ (HBHI) approach – a targeted malaria response in all 11 high malaria burden countries, and India is one such country outside Africa. **Figure 9** shows how HBHI countries have initiated high-level political engagement and support [5]. In a recent virtual meeting of MPAC of WHO commended the efforts made so far in the COVID-19 pandemic. Training should aim to increase the sub-national capacity for evidence-driven decision-making and translating those decisions into actions [13].

Over 100 countries have successfully eliminated malaria in the last century. In the past two decades a lot of initiatives have been launched to contain and eliminate malaria, viz., Roll Back Malaria (RBM), President’s Malaria Initiative (PMI), Asia Pacific Malaria Elimination Network (APMEN), E-2020, Malaria Elimination Research Alliance (MERA) - India [84, 85]. Many countries have developed national elimination goals. Regional networks have been formed to facilitate collaboration [86]. Leaders from the Asia Pacific Leaders Malaria Alliance (APLMA) in November 2014 and the African Leaders Malaria Alliance in January 2015 endorsed regional goals for malaria elimination by 2030 [87, 88]. In 2009, the APMEN was established initially in 10 countries (Bhutan, China, Democratic People’s Republic of Korea (DPR Korea), Indonesia, Malaysia, the Philippines, Republic of Korea, Solomon Islands, Sri Lanka, and Vanuatu), and now have expanded to 18 countries adding Bangladesh, Cambodia, Lao People’s Democratic Republic (Lao PDR), India,

Indicator	Americas and Caribbean	South Asia and East Africa and Pacific	Europe and Central Asia	Middle East and North Africa	Sub-Saharan Africa	Total
Total number of Countries	46	38	58	23	45	211
1900	2	13	3	1	1	20
1900–1949	0	0	9	0	0	9
1950–1978	23	5	35	4	1	68
1979–1990	0	1	2	2	1	6
1991–2019	4	2	9	7	0	22
Total number of malaria-free countries	29	21	58	14	3	125

Table 1. Number of countries and territories that eliminated malaria by region, 1900–2019. Compiled from Shretta et al. [84] and WHO [8].

Figure 9.
Schematic presentation how ‘High burden to high impact’ countries get back on track to achieve the GTS 2015 milestone. Source WHO. [5].

Nepal, Papua New Guinea, Thailand, and Vietnam. Among these, India contributes maximum malaria cases explaining the importance of malaria elimination in this country [89]. But China (Yunnan), Cambodia, Thailand, Myanmar, Lao PDR and Viet Nam in Greater Mekong Subregion greatly reduced falciparum malaria [85] and now the Mekong Malaria Elimination Programme is ready for the last mile of malaria elimination [90].

The real malaria burden lies in the sub-Saharan Africa region. There were significant impact of malaria control from 2000 to 2015, but the situation has plateaued between 2015 and 2019 largely attributed to emerging synthetic pyrethroid (PY) resistance in the principal malaria vector *An. gambiae*. Adding piperonyl butoxide (PBO) enhances the efficacy of PY, but the requirement is huge, and costs have risen substantially amidst financial gap. In high insecticide resistance areas, pyrethroid-PBO nets increase mosquito mortality and reduce blood feeding rates that leads to lower malaria prevalence. But the impact of pyrethroid-PBO LLINs on mosquito mortality was not sustained over 20 washes. There is a little evidence to support higher entomological efficacy of pyrethroid-PBO nets in areas where the mosquitoes show lower levels of resistance to pyrethroids [91]. This warrants routine monitoring of insecticide resistance to take appropriate decisions by the national programme managers.

In India the total global burden has reduced from 4% in 2018 to 2% in 2019 of total malaria cases. But it still falls under the ‘high burden to high impact’ countries outside Africa. India contributed 87.9% of total malaria cases and 86% of malaria deaths in the South East Asia region of WHO [5]. This requires a special attention for the high burden states with high *P. falciparum* cases. For example, malaria cases in Karnataka state, India fell 98.95% in 2019 compared with 1995 data, but 70 to 80% of reported cases from Mangalore city alone requires concerted efforts. Now, a special action plan with digital surveillance has been initiated for the last five years, which is showing some results. Hopefully, Karnataka can declare malaria elimination by 2025 ahead of India’s deadline of 2027 [81, 82]. Such strong surveillance system can be implemented to find a solution to get freedom from malaria.

In recent years many innovative intervention strategies have been shown to be promising. Finding the reasons for *Pfhrp2/3* deletions and drug resistance in *P. falciparum* is an important step to address this major issue. BV1 lineage is associated

with these two important issues [8]. Moreover use of other more advanced RDT like malaria-RDT (mRDT) can be an alternative where deletions *Pfhrp2/3* are low [92]. However, alternative RDT options based on detection of the *Plasmodium* lactate dehydrogenase [pLDH]) are limited; in particular, there are currently no WHO-prequalified non-*Pfhrp2* combination tests that can detect and distinguish between *P. falciparum* and *P. vivax* [5]. In such situation photo-induced electron transfer- polymerase chain reaction (PET-PCT) or microPCR devices can be applied with highest accuracy at point-of-care. Issue of asymptomatic malaria cases can be addressed because these devices can detect a very low level of parasitaemia [93, 94].

Besides, burden of asymptomatic parasite cases in most malaria-endemic countries, molecular monitoring of drug resistance in parasites and insecticide resistance in vector populations are two vital parameters which can help make correct policy decisions by the national malaria control programmes. Many countries have initiated for molecular surveillance; for example in Haiti [93], in Asia-Pacific countries [95] and Cambodia [96]. Genome analysis is not done in most situations due to its prohibitive cost. Now very sensitive and low-cost oxford nanopore platform is very useful and many researchers are using this to find out the gene flow and genetic diversity in parasites and vectors [97]. SNP barcode is used instead of microsatellite technology using this platform [98]. Scientists in Africa have been working on these issues on Pathogens Genomic Diversity Network Africa [99]. Such platforms must work on other regions also to provide guidance to the national malaria programme from time to time for taking corrective measures to change the policy decisions. Similarly, vector resistance to insecticide pattern can also be established to take correct selection of effective insecticide [100].

Monitoring of insecticide resistance allows targeting of specific interventions with pyrethroid-PBO nets, and resistance mechanisms finding mixed-function oxidase (MFO) resistance mechanisms over time. Such monitoring also enables programmes to assess the value of different insecticide resistance management strategies like IRS rotation, new types of ITNs and LLINs or other effective tools. Using genotyping to detect insecticide resistance is quicker to implement than phenotypic assays that require rearing of larvae, even wild type adults can be used when available in sufficient numbers. It is possible that resistance could be underestimated due to unknown age of the mosquito. With this approach, shifts in allele frequencies may be easier to detect than shifts in phenotype over short time periods [13].

Finding origin of the parasites and gene flow in the elimination era is a challenging task. Genetic relatedness studies using metrics of identity-by-state (IBS) and identical-by-descent (IBD) - alleles that are genetically the same, and alleles that come from a common ancestor, respectively can address this issue [101]. This requires a number of informative markers (molecular barcode genotyping) that vary depending on the level of transmission in the geographic area under examination. A barcode is considered informative for relatedness by IBS at >0.95 relatedness. When using this measure in a low transmission setting, relatedness can serve as a key indicator for distinguishing imported and local transmission and understanding the persistence of transmission in the area [13].

In high *falciparum* areas an alternative approach to understanding receptivity risk for imported and onward indigenous transmission of malaria is to investigate parasite markers in parasite-vector interactions that determine whether the parasite can successfully infect the mosquito. *Pfs47* is a target of interest allows the ookinete to evade the immune response of the mosquito midgut and successfully develop into an oocyst. The allele is polymorphic with signatures of natural selection relevant to the geographic origin of the parasite. *P. falciparum* isolates are more compatible with *Anopheles* species from their region of origin. *Pfs47* single nucleotide polymorphisms (SNPs) can therefore be used to predict

the transmission risk of imported *P. falciparum* and help establish its geographic origin. Specific SNPs for vivax malaria is warranted [13].

Now time has come act judiciously to eliminate malaria at subnational to achieve elimination target at national level. Again surveillance that can capture and report individual cases in time to investigate and take action [81, 82]. The Chinese national malaria elimination programme, now approaching WHO certification used effectively in subnational initiatives to interrupt malaria transmission followed by validations of elimination [102]. Similarly, Kenya has established a national strategic action plan 2019–2023 to achieve malaria elimination in targeted countries by 2030 [103]. Malaysia has successfully launched malaria elimination partnering between the public and commercial sectors in Sabah [104], but the rising threat of zoonotic *P. knowlesi* is a matter of concern [105].

Time has come to look back the success stories of malaria elimination efforts. In October 1998, the Director-General of WHO launched the RBM initiative. It was established through a partnership between WHO, the World Bank, the United Nations Children's Fund (UNICEF) and the United Nations Development Programme (UNDP) [106]. The purpose of launching RBM initiative was in this direction. In Karnataka, India we successfully implemented larvivorous fish-based malaria elimination campaign [107, 108]. We need to repurpose the larval source management strategy. The best historical example is the successful eradication of accidentally introduced African vector *Anopheles gambiae* in 54000 km² of largely ideal habitat in Northeast Brazil (Natal) from Dakar, Senegal in the 1930s and early 1940s. This outstanding success was achieved through an integrated programme but relied overwhelmingly upon larval control. The success of Frederick Lowe Soper and Rockefeller Foundation's International Health Division campaigned with anti-larval chemical Paris Green and eliminated *An. gambiae* before the scheduled timeline [62]. It was a significant watershed in the history of malaria control, and revived the faith in vector control strategies that paved the way for the application of eradication methods in the fight against malaria following World War II. This experience was soon repeated in Egypt and another larval control programme successfully suppressed malaria for over 20 years around a Zambian copper mine [109]. It is important to revisit all such success stories implemented earlier. Today, with the development of advanced technology, we have more options like application of a new anti-larval product Aquatain AMF™ for use in LSM. It is a silicon-based liquid formulation that forms a very thin film on stagnant water. The mosquito larvae are killed due to physical and mechanical action. Drone technology are now used for anti-larval applications [110]. Rhamnolipids – a class of glycolipid may be applied as anti-larval agent especially against *Ae. aegypti* and may be against *A. stephensi* [111].

Efforts are also being made to develop technology for diagnosis of malaria parasites and identification of vector mosquitoes using artificial intelligence and machine learning. [112, 113]. Aptamer technology can be applied which has the potential to revolutionize biological diagnostics and therapeutics. This technology can be used for malaria diagnosis in place of HRP2 which is facing certain problems [114, 115]. This also be used in malaria as adjunct therapy [116]. Other most prospective gene editing technology in vector mosquitoes especially in *An. gambiae* in Africa where most malaria exists, and also in *An. stephensi* – the most invasive malaria vector. [61, 110, 117]. It is important to find new drug molecules to counter drug resistance challenge in parasites. Medicines for Malaria Venture (MMV) has been working for more than two decades in this front. *P. vivax* liver stage assays platform was initiated by MMV to discover new molecules for anti-relapse drugs to work against hypnozoites. India was one of the partners under this initiative [118]. A new vaccine candidate R21 with adjuvant Matrix-M™ developed by Novamax has shown a new hope for an effective vaccine that could be used in malaria

elimination. A Phase 2B trial on 450 children aged 5-17 months in Burkina Faso, West Africa showed 77% efficacy [119].

Now, all resources have been diverted to COVID-19 even WHO alerted in the beginning of the pandemic. In a recent article published in *Nature* emphasized the need to create a general attention like COVID-19 to all tropical diseases including malaria. The author has expressed concerns about the reversing the impact on malaria elimination achieved so far especially in Sub-Saharan Africa [120]. It may be mentioned that in March 2020, as the COVID-19 pandemic spread rapidly around the globe, WHO convened a cross-partner effort to mitigate the negative impact of the corona virus in malaria-affected countries and contribute to the COVID-19 response otherwise, much of the progress against malaria was under enormous risk, with the potential to wipe out 20 years of malaria gains [120].

Funding in malaria elimination efforts is an important component. Sufficient and timely release of allocated funds would ease out many constraints for which strict financial management is utmost necessary. The total annual financial resources needed were estimated at US\$ 4.1 billion in 2016, rising to US\$ 6.8 billion in 2020 to achieve the GTS milestone. An additional funding of US\$ 0.72 billion is estimated to be required annually for global malaria research and development (R&D). In 2019, the total funding for malaria control and elimination was estimated at US\$ 3.0 billion, compared with US\$ 2.7 billion in 2018 and US\$ 3.2 billion in 2017. The amount invested in 2019 falls short of the US\$ 5.6 billion estimated to be required globally. In recent years, the funding gap between the amount invested and the resources needed has widened dramatically, increasing from US\$ 1.3 billion in 2017 to US\$ 2.3 billion in 2018, and to US\$ 2.6 billion in 2019 [5].

9. Conclusion

In the renewed efforts to eliminate malaria more needs to be done following the strategies and technologies adopted by those countries successfully eliminate malaria. High burden to high impact countries need special attentions. If these countries in Africa and India are free from malaria over 80% of malaria burden can be curtailed. There are several roadblocks namely gene deletions in *Pfhrp2/3*, insecticide resistance, drug resistance in *P. falciparum* and also in *P. vivax* need to be addressed following new technologies like gene editing and Aptamar technologies. There are many gaps in surveillance for which smart digital surveillance is an important strategy that need to be implemented on priority. Artificial intelligence and machine learning should find proper place to solve many ongoing problems of diagnosis and effective implementation, monitoring of the elimination programme. Routine malaria molecular surveillance of parasites and vectors at subnational and regional levels must be carried out to take correct and appropriate measures policy decision makers. As long distance night travel and invasiveness of vector mosquitoes have been established or otherwise, LSM must find priority. Like COVID-19, other tropical diseases like malaria must be given priority with proper funding provisions.

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Conflict of interest

The authors declare no conflict of interest.

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