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Malaria in the Greater Mekong Subregion: Heterogeneity and Complexity

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Abstract

The Greater Mekong Subregion (GMS), comprised of six countries including Cambodia, China's Yunnan Province, Lao PDR, Myanmar (Burma), Thailand and Vietnam, is one of the most threatening foci of malaria. Since the initiation of the WHO's Mekong Malaria Program a decade ago, malaria situation in the GMS has greatly improved, reflected in the continuous decline in annual malaria incidence and deaths. However, as many nations are moving towards malaria elimination, the GMS nations still face great challenges. Malaria epidemiology in this region exhibits enormous geographical heterogeneity with Myanmar and Cambodia remaining high-burden countries. Within each country, malaria distribution is also patchy, exemplified by 'border malaria' and 'forest malaria' with high transmission occurring along international borders and in forests or forest fringes, respectively. 'Border malaria' is extremely difficult to monitor, and

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frequent malaria introductions by migratory human populations constitute a major threat to neighboring, malaria-eliminating countries. Therefore, coordination between neighboring countries is essential for malaria elimination from the entire region. In addition to these operational difficulties, malaria control in the GMS also encounters several technological challenges. Contemporary malaria control measures rely heavily on effective chemotherapy and insecticide control of vector mosquitoes. However, the spread of multidrug resistance and potential emergence of artemisinin resistance in *Plasmodium falciparum* make resistance management a high priority in the GMS. This situation is further worsened by the circulation of counterfeit and substandard artemisinin-related drugs. In most endemic areas of the GMS, *P. falciparum* and *P. vivax* coexist, and in recent malaria control history, *P. vivax* has demonstrated remarkable resilience to control measures. Deployment of the only registered drug (primaquine) for the radical cure of vivax malaria is severely undermined due to high prevalence of glucose-6-phosphate dehydrogenase deficiency in target human populations. In the GMS, the dramatically different ecologies, diverse vector systems, and insecticide resistance render traditional mosquito control less efficient. Here we attempt to review the changing malaria epidemiology in the GMS, analyze the vector systems and patterns of malaria transmission, and identify the major challenges the malaria control community faces on its way to malaria elimination.

Keywords

malaria; the Greater Mekong Subregion; epidemiology; *Anopheles* vectors; drug resistance; border malaria; elimination

1. Introduction

According to World Malaria Report 2010, the estimated annual malaria incidence for 2009 was 225 million cases, resulting in ~781,000 deaths (WHO, 2010b). While most of the malaria burden is in sub-Saharan Africa, Southeast (SE) Asia accounted for 10% of the global malaria morbidity and 5% of the global mortality in 2008. Since the launch of the Roll Back Malaria Initiative by WHO in 1998, malaria control has intensified in endemic countries, supported by better financial support and technological development. A Global Malaria Action Plan seeks to eliminate malaria using integrated approaches including vaccines, insecticide-treated mosquito nets (ITNs), indoor insecticide residue spray (IRS), and improved drug treatments (<http://www.rollbackmalaria.org/gmap/index.html>). Once again, malaria eradication is on the agenda of the international community (Feachem and Sabot, 2008; Roberts and Enserink, 2007). Of the 99 malaria endemic countries, 32 have declared a national policy of malaria elimination (Feachem et al., 2010).

Within SE Asia, the Greater Mekong Subregion (GMS) has been one of the most dangerous foci of malaria. The GMS is comprised of Cambodia, China (Yunnan province), Lao PDR, Myanmar (Burma), Thailand, and Vietnam, which vary in political structure, socio-economic and financial resources, public health system, and disease ecology. The GMS is one of the most densely populated areas with topographical environments ranging from coastal plains to river estuaries and rugged mountainous terrains. Such divergent ecological systems offer diverse breeding habitats for multiple mosquito vector species with distinctive preferences for forest edges, foothills, or agricultural fields. Climates range from temperate conditions with disrupted malaria transmission during the harsh winter to tropical climates where malaria transmission occurs year round. The Mekong River runs through all six countries, and its watershed plays an important role in the transmission of vector-borne diseases. As such, malaria epidemiology in this region is complex, characterized by immense geographical heterogeneity in disease distribution with many areas of high endemicity, differential prevalence of the two most predominant parasites *Plasmodium*

falciparum and *P. vivax*, which require different drug treatments, and diverse vector systems with different vectorial capacities for these parasites (Socheat *et al.*, 2003). Furthermore, the GMS harbors the epicenter of multidrug resistant (MDR) *P. falciparum* in the border area between Cambodia and Thailand, which is gradually encompassing the tropical world (Hastings, 2004; Wongsrichanalai *et al.*, 2002). Recent detection of artemisinin resistance in the same area represents a regional and global emergency (Dondorp *et al.*, 2009; Noedl *et al.*, 2008). Therefore, malaria control in the GMS is not only important for the immediate region, but also for global malaria control (WHO, 2011).

2. Malaria situation in the GMS: evidence of changing epidemiology

2.1. Overview

Like other malaria-endemic regions of the world, the GMS has witnessed dramatic changes in its malaria situation. Since malaria is generally a disease of the poor, the history of malaria, to a large extent, mirrors the broader political environments and economic evolution in this region. Between 1950s and 1990s, systematic organizational efforts saw the gradual elimination or near eradication of major malaria foci from the central plain regions of several nations in this region, only to see its gradual rise in Cambodia, Myanmar, Vietnam, Thailand, and China due to various precipitating events and conditions. Although regional political and economic instability is partially blamed for the resurgence of malaria, human population expansion and mobility into forested regions, and environmental changes such as urbanization and deforestation have all contributed to the changing picture of malaria epidemiology (Kidson *et al.*, 2000). Perhaps, the single most important culprit responsible for the regional and global malaria resurgence is the emergence and spread of MDR *falciparum* malaria from the GMS. In recent years, the malaria control and elimination campaign faces another dreadful threat: the emergence of artemisinin resistance (Dondorp *et al.*, 2009; Noedl *et al.*, 2008). Therefore, containment of the resistant parasites has been and will remain the focus as well as the greatest challenge of malaria control efforts. Lessons from malaria control history in this region have taught us that the combination of community organization, exhaustive case detection, management and focus elimination, and strong political will at all levels of the society makes elimination of malaria from large areas possible. Yet, sustainability of elimination requires collaborative political determination of individual nations in this region. In recognition of the need for coordinated efforts, WHO launched the Mekong Malaria Program in 1999 with the aims of significantly reducing malaria-associated morbidity and mortality in this region and curbing the spread of MDR *P. falciparum*. Since the inception of this program, malaria control in the GMS has made a huge stride, accompanied by the continuous decline in annual malaria incidence and deaths (Delacollette *et al.*, 2009; Singhasivanon, 1999; Socheat *et al.*, 2003). It was estimated that there was a 25% reduction in the number of confirmed malaria cases and 60% reduction in malaria-associated mortality in the GMS from 1998 to 2007 (Delacollette *et al.*, 2009). Multiple factors, including stronger political will of the governments, increasing investments in malaria interventions, and strengthening public health systems have all contributed to these achievements. Yet, even greater challenges have been laid in front of the malaria control community. Currently, two countries in the GMS, Myanmar and Cambodia, are still among the countries with highest malaria-burden in the world (Fig. 1) (WHO, 2010b). In particular, the number of malaria cases and malaria-induced mortality in SE Asia has consistently been the highest in Myanmar for the past three decades.

2.2. Country-specific epidemiology and drug resistance

Cambodia—Cambodia had 80,644 reported malaria cases in 2008, and this figure could be significantly higher if malaria cases treated in the private sector were taken into account. Malaria incidence is the highest in the eastern province of Mondulhiri, the most sparsely

populated province. In the western region that borders Thailand, malaria incidence is also high, largely due to the location of the high-risk groups such as migrant workers clearing forests for land. The Thai-Cambodian border has been an epicenter of antimalarial drug resistance, and parasites with resistance to chloroquine (CQ) and antifolates have emerged here and spread to the rest of the world (Anderson and Roper, 2005; Roper et al., 2004; Wootton et al., 2002). The emergence of MDR parasites is the most worrisome, since such parasites may develop resistance to new antimalarial drugs at an accelerated rate (Rathod *et al.*, 1997). It is therefore not surprising that parasites with reduced sensitivity to artemisinin and related drugs have recently been detected in this area (Dondorp et al., 2009; Noedl et al., 2009, 2010). Recently, WHO has developed a five-point action plan that aims to contain the artemisinin-tolerant parasites in this region (WHO, 2009, 2011).

China—Since its launch in 1955, China's national malaria control program (NMCP) has savoured great success. Prior to its implementation, annual malaria incidence totalled 6.79 million cases in 1954, accounting for over 60% of recorded acute infectious diseases (Yip, 1998). Despite two major outbreaks in the 1960s and 1970s, the country saw a steady decrease in the number of cases, especially after the reinstatement of systematic control efforts beginning in 1978 (Tang et al., 1991). By the year 2000, malaria cases dropped to only 29,039 reported cases. It is noteworthy to mention that case underreporting may have been high, suggesting significant underestimation of the disease burden (Zhang, 2005; Zhang et al., 2004). From the most recent malaria distribution map in 2008, 110 counties had annual malaria incidence rate exceeding 1/10,000. Most of these counties were located in three areas: central China, Hainan Island, and Yunnan Province (Fig. 1). Among all malaria cases, vivax malaria accounted for more than 80%, whereas autochthonous falciparum malaria only occurred in subtropical Hainan and Yunnan provinces, which have been historically the most malarious regions in China (Sheng *et al.*, 2003). In addition, the major proportion of falciparum cases happened in young adult males of 15-50 years of age (Lin et al., 2009), partially attributed to the higher mobility and outdoor activities of this group. On Hainan Island, malaria transmission peaks in May to October and is distributed in the hilly, forested south-central counties of the island (Xiao et al., 2010). Similarly, malaria incidences in Yunnan are temporally and spatially clustered (Clements et al., 2009; Hui et al., 2009). Interestingly, before the year 2000, a single malaria incidence peak was observed annually from June through August, whereas in 2000-2005 two malaria peaks occurred annually, one in May-July and one in October-November. As expected, high-incidence counties are clustered at international border areas and overlap with the poverty map of Yunnan, suggesting a correlation with socio-economic status (Clements et al., 2009; Hui et al., 2009). Yunnan has more than 4000 km of border line with three malarious countries, Myanmar, Laos, and Vietnam, and imported cases accounted for a large proportion of adult infections (Lin et al., 2009). Furthermore, the coexistence of *P. falciparum* and *P. vivax* in Yunnan makes disease treatment challenging. Although artemisinin-based combination therapy (ACT) is generally effective in treating *P. falciparum*, there is an indication of reduced sensitivity of parasites to artemisinin and its derivatives (Yang *et al.*, 2003). As Yunnan has the longest history of artemisinin usage mostly in the form of monotherapy, rigorous clinical efficacy studies are warranted in this region. In central China, malaria is characterized by temperate-zone *P. vivax* with a long relapse interval. From 2000 to 2006, malaria incidence in the central provinces (Anhui, Henan, Hunan, Hubei, and Jiangsu) rose steadily, contributing significantly to nation-wide malaria resurgence (Sleigh et al., 1998; Xu et al., 2006; Zhou et al., 2007). Spatially, malaria in this region is clustered near the Huai River (Zhang et al., 2008). Although environmental changes may be largely responsible for the resurging vivax malaria in central China, other factors such as drug resistance may also be held accountable. The clinical efficacy of the CQ-primaquine combination has not been evaluated in central China, but a recent study at the Yunnan-Myanmar border suggested

reduced efficacy of this drug combination for treating vivax malaria (Liang et al., 2009). Since China is embarking on malaria elimination, vivax malaria in central China, prevention of malaria reintroduction, and management of drug resistance are among the major obstacles.

Lao PDR—In recent years, the malaria situation in Lao PDR has notably improved, as shown by the reduction in number of malaria cases and malaria deaths. However, it is noteworthy that the epidemiology data are based on hospital records, which inevitably underestimate the actual number of malaria cases, since the rate of self-medication is high. Despite great progress made in malaria control, malaria continues to be a serious public health problem in southern areas of the country, particularly in remote areas (Delacollette et al., 2009; Socheat et al., 2003; WHO, 2008a). Chloroquine and sulfadoxine-pyrimethamine (SP) are no longer viable therapy for falciparum malaria, since resistance to these drugs is widespread in this country (Mayxay et al., 2003, 2007). Currently ACTs have excellent efficacy, but widespread availability of substandard and counterfeit antimalarials is a serious problem and threat to the management of drug resistance.

Myanmar—The malaria burden in Myanmar is the heaviest among the GMS nations. More than half of the malaria cases and ~75% of the malaria deaths in the GMS in 2007 occurred in Myanmar (WHO, 2008a). The annual malaria incidence in Myanmar decreased from 1998 to 2007. Despite this progress, investments in malaria control are moderate compared to other GMS countries. In 2008, malaria incidence rose again from 9.0% to 10.8%. In 2009, 49.3% of the cases occurred in forest and forest-fringe areas of Kachin, Rakhine states and Sagaing Division; 74% of reported cases were from *P. falciparum* infections. In the border areas, where poor ethnic minorities (e.g., Wa, Dai, Lahu, Karen, etc.) are concentrated, malaria burden is particularly high and malaria outbreaks occur frequently (WHO, 2008b). For example, malaria outbreaks in November of 2003 in Shan and Kachin states resulted in the death of nearly a thousand people (Li et al., 2005). Kachin State also had the highest malaria-related mortality rate, with 7.8 deaths/1,000 people in 2005 (WHO, 2008a). Public health infrastructure in the remote states is poor and reported cases may severely underestimate the disease burden. Drug resistance management also makes up an important challenge in this country. Resistance to CQ and SP is widespread, whereas resistance to other antimalarials such as mefloquine and quinine is also on the rise (Meng et al., 2010). Another important impact of the malaria situation in Myanmar is its contribution to malaria in neighboring countries, which seriously impedes the progress toward the goal of malaria elimination in these countries (Beyrer and Lee, 2008).

Thailand—Since the implementation of the national malaria control program in the 1950s, malaria associated morbidity and mortality have been reduced dramatically in Thailand. Like other countries in this region, malaria displays significant geographical heterogeneity and is exemplified by the “border malaria” type, with most of the malaria cases concentrated along the borders with Myanmar and Cambodia (Childs et al., 2006; Zhou et al., 2005). Many of the border regions are forested with heavy population migrations, and non-Thais, mostly migrant workers from the neighboring countries, make a disproportionate contribution to the regional malaria burden. In addition, political unrest among the minorities in Myanmar has led to significant cross-border population movements, and displaced minority populations are at increased risks of malaria (Richards et al., 2009). Moreover, certain life style and behavioral factors of the hill tribes and migrant workers are also associated with increased risks of malaria infection (Chaveepojnkamjorn and Pichainarong, 2004, 2005; Pichainarong and Chaveepojnkamjorn, 2004). Malaria in Thailand shows a two-peak annual seasonality, often following the patterns of rainfall (Childs et al., 2006; Cui et al., 2003). The predominant parasite species are *P. falciparum*

and *P. vivax*, and *P. vivax* has become more prevalent than *P. falciparum* at the turn of the century (Sattabongkot *et al.*, 2004). The other two human malaria species, *P. malariae* and *P. ovale*, are also present at relatively high levels, but are frequently overlooked due to limited sensitivity of the detection methods (Zhou *et al.*, 1998). Furthermore, sporadic zoonotic infections by the monkey parasite *P. knowlesi* were also reported (Jongwutiwes *et al.*, 2004; Putaporntip *et al.*, 2009a). In recent years, there is also a trend of changing malaria epidemiology in Thailand. In some southern provinces, such as Yala and Narathiwat where malaria was largely eliminated decades ago, it has recently reappeared with sporadic outbreaks occurring in several districts. Molecular population genetic studies revealed that the genetic structure of the parasites, in contrast to being panmictic in the west, is more clonal in the south and likely represents recent expansion of a limited number of parasite strains (Putaporntip *et al.*, 2009a, b). In eastern areas of Thailand, such as Sa Kaeo Province, *P. vivax* has replaced the dominant status of falciparum malaria with its relative prevalence increasing from <25% before 1995 to almost 90% in recent years. A noticeable change in vector species composition and abundance is a plausible reason for the shift in parasite species prevalence (Limrat *et al.*, 2001), but this change in malaria epidemiology still awaits for further investigations. The high proportion of MDR *P. falciparum* parasites near the borders constitutes another big challenge for the control program. Antimalarial drug policy has changed many times in the past in response to the escalating problem of drug resistance (Sattabongkot *et al.*, 2004). Since the switch of the national drug policy to artesunate plus mefloquine in the early 1990s, clinical and parasitological responses to this ACT regimen have dropped considerably (Carrara *et al.*, 2009; WHO, 2008a). This problem is especially serious at the Thai-Cambodian border (Wongsrichanalai and Meshnick, 2008), where measures to contain potential artemisinin-resistant parasite strains are being deployed (WHO, 2009, 2011).

Vietnam—Prior to the early 1990s, malaria was a major cause of morbidity and mortality in Vietnam. As the result of improved socio-economic conditions, increased government investment, and community-based monitoring, the malaria burden in Vietnam has been dramatically alleviated in recent years (Thang *et al.*, 2009). Using a combination of control strategies such as ITNs and early diagnosis and treatment of symptomatic patients (Hung le *et al.*, 2002), the number of malaria cases in Vietnam has plunged to merely 11355 in 2008 (Manh *et al.*, 2011). Much like the situation in Laos, malaria in Vietnam is distributed in the central and southern parts of the country, and malaria occurs mostly in the forests or forest edges (Erhart *et al.*, 2005; Sanh *et al.*, 2008). *Plasmodium falciparum* remains the predominant species, whereas infections with the monkey malaria parasite *P. knowlesi* have also been detected in children in the central region (Van den Eede *et al.*, 2009). Because of the switching of treatment policy from CQ to ACTs, the prevalence of the pfcr1 76T mutation has dropped, suggesting increased sensitivity to CQ. However, the prevalence of antifolate resistance mutations remains high (Isozumi *et al.*, 2010). Longitudinal surveys of artemisinin resistance suggest that *P. falciparum* parasites are still highly sensitive to the ACTs in this region (Thanh *et al.*, 2010), but vigorous clinical studies may be needed to verify such claims.

3. Malaria vectors in the GMS

3.1. Diversity in vector species

One distinct feature of malaria transmission in the GMS is the diversity in vector species and the tremendous spatial heterogeneity in distribution patterns (WHO, 2007a). Figure 2 shows the distribution of reported malaria vector species in the GMS. Generally, members of *An. minimus*, and *An. dirus* species complexes, as well as *An. sinensis* are the most important vectors, but the importance of each species in malaria transmission varies greatly among

regions. For example, in tropical and subtropical regions of China (below 25°N latitude), members of *An. minimus* complex and the *An. dirus* complex are the main vectors, whereas in more temperate regions (above 33°N latitude), *An. sinensis* is the major malaria vector (Chareonviriyaphap et al., 2000, 2003; Lee et al., 2001; Pinichpongse and Bullner, 1967). In the areas between 25°N latitude and 33°N latitude, *An. lesteri* is an important malaria vector. In Thailand, *An. dirus* complex, *An. minimus* complex, *An. aconitus*, *A. sundaicus* complex are the main malaria vectors. *Anopheles baimaii* and *An. pseudowillmori* of the *An. maculatus* group are considered malaria vectors with local importance (Chareonviriyaphap et al., 1999). Furthermore, malaria vector species composition varies even among different localities in a region because vector species exhibit different requirements for larval habitat. For example, *An. dirus* and *An. baimaii* are often found in stagnant and shaded waters in forests (Oo et al., 2003) whereas *An. minimus* complex generally uses habitats along streams in hilly areas (Overgaard et al., 2002), and *An. sinensis* is often found in rice fields and irrigation canals (Rueda et al., 2006).

3.2. Species complex and molecular identification

It must be noted that many malaria vectors in the region belong to species complexes and groups (Harbach, 2004), which include closely related species that are difficult to distinguish morphologically, yet often differ in bionomics and capacity for malaria transmission (Manguin et al., 2008). For example, *An. hyrcanus* complex includes several important but morphologically similar malaria vector species such as *An. sinensis*, *An. lesteri* (syn. *Anthropophagus*) (WHO, 2007a). The sympatric occurrence of multiple morphologically indistinguishable or misidentified vector species complicates our understanding of the roles of individual vector species in malaria transmission and hampers our ability to optimize vector control strategies. In this regard, accurate mosquito identification becomes particularly important. The use of molecular methods has greatly improved the accuracy of species identification. PCR-based methods have the advantage of requiring minute amounts of material for analysis, and ribosomal DNA (rDNA) has been a popular choice for differentiating *Anopheles* species (Collins and Paskewitz, 1996). This is because the rDNA units are typically present in hundreds of copies per genome making them particularly easy to amplify, and the internal transcribed spacers (ITSs) separating ribosomal RNA genes generally exhibit variations among the sibling species (Alves et al., 2005). By designing species-specific primers in the ITS2 region, Walton and her colleagues have developed a PCR-based identification method to distinguish five species of the *An. dirus* complex (*An. nemophilous*, *An. dirus* [=species A], *An. cracens* [=species B], *An. scanloni* [=species C], and *An. baimaii* [=species D]) from Thailand (Obsomer et al., 2007; Walton et al., 1999), four species of the *An. annularis* group (*An. annularis*, *An. nivipes*, *An. philippinensis*, and *An. pallidus*) (Walton et al., 2007a), and five species of the *An. maculatus* group (*An. maculatus*, *An. dravidicus*, *An. pseudowillmori*, *An. sawadwongporni* and species K) (Morgan et al., 2009; Walton et al., 2007b). Similarly, the PCR-based identification method was also used to distinguish mosquitoes of the *An. maculatus* group in China (Ma et al., 2006). Phuc et al. (2003) reported a multiplex PCR assay based on ITS2 sequences to identify *An. minimus* and *An. harrisoni* (=species C) of the *An. minimus* complex (Green et al., 1990; Phuc et al., 2003). Gao et al. (2004) reported a PCR-RFLP method for discriminating *An. lesteri* and *An. sinensis* (Gao et al., 2004).

In addition to the PCR-based methods, another potentially useful method for molecular species identification is the loop-mediated isothermal amplification (LAMP) technique (Parida et al., 2008). LAMP is a one step nucleic acid amplification that relies on autocycling strand-displacement DNA synthesis. It is performed under isothermal conditions using a DNA polymerase with strand displacement activity. Moreover, the amplification products can be visualized directly (Tomita et al., 2008). The LAMP method offers high

sensitivity, requires simple equipment (e.g., a water bath), and no electrophoresis, and thus the method may be suitable for field settings where sophisticated equipment is lacking.

3.3. Insecticide resistance

Currently, long-lasting insecticide treated nets (LLINs) and IRS are the primary vector control tools in the GMS. The insecticides of choice for bednet impregnation and IRS are pyrethroids because of their high efficacy, rapid rate of knockdown, strong mosquito exito-repellency, and low mammalian toxicity. Synthetic pyrethroids, particularly permethrin and deltamethrin, are widely used throughout the world. Reducing vector-human contact through the use of LLINs has been shown to be effective in reducing malaria prevalence in SE Asia (Sexton, 1994; Li et al., 1989). Along with the use of insecticides to reduce abundance of disease vectors, the application of insecticides for agricultural purposes also increases the likelihood and speed at which resistance can develop (Overgaard et al., 2005). Previous studies in SE Asia suggest a patchy distribution of insecticide resistance in four malaria vector species. Table 2 summarizes the reported resistance to DDT and pyrethroid insecticides in the GMS. Between 1990 and 1997, DDT resistance was detected in *An. dirus s.l.* and *An. minimus s.l.*, and permethrin resistance was found in a population of *An. minimus s.l.* from northern Thailand (Chareonviriyahpap et al., 1999). In Vietnam, pyrethroid-susceptible and tolerant *An. minimus* populations were found, and *An. minimus* also showed resistance to DDT and pyrethroids in some sites in Cambodia and Laos (Prapanthadara et al., 2000; Van Bortel et al., 2008). *Anopheles dirus s.s.*, the main vector in forested malaria foci, is permethrin susceptible throughout the GMS, but in central Vietnam it shows possible resistance to pyrethroids (Van Bortel et al., 2008). Resistance to deltamethrin was reported in *An. sinensis* in China (Cui et al., 2006). To our knowledge, there is no information on the status of insecticide resistance in malaria vector species in Myanmar.

Pyrethroid resistance in the malaria vector and other insects was found to involve two mechanisms: a) a mutation in the target site, region II of the *para*-type sodium channel gene, causing a change in affinity between the insecticide and its binding site on the sodium channel, a phenotype known as knockdown resistance (*kdr*) (Martinez-Torres et al., 1998; Ranson et al., 2000), and b) increased rates of detoxification through elevated levels of P450 monooxygenase, esterase, and glutathione *S*-transferases (Brooke et al., 2001). A recent study by Verhaeghen and colleagues did not detect any *kdr* alleles in *An. minimus* and *An. dirus* in Vietnam, Laos, Cambodia, and Thailand (Verhaeghen et al., 2009) (Table 2). Rather, pyrethroid detoxification may be an important resistance mechanism in these species. It is entirely possible that the extent by which the two mechanisms (mutations in the target site and increased detoxification) confer pyrethroid resistance is different across vector species or across different localities. Although pyrethroid resistance in SE Asia is by far not as serious an issue as it is in Africa, continuous surveillance is necessary for sustained control of vectors.

4. Malaria elimination: are we ready for this challenge?

For countries with low malaria endemicity, WHO has proposed a four-phased plan for malaria elimination (WHO, 2007b). WHO defines malaria elimination as “the interruption of local malaria transmission in a defined geographical area, creating a zero incidence of locally contracted cases. Imported cases will continue to occur and continued intervention measures are required”. A three-part strategy proposed to shrink the malaria map is now widely accepted, which includes 1) aggressive control in the malaria heartland, 2) progressive elimination of malaria from endemic margins, and 3) research to bring forward new tools (Feachem, 2009). The current malaria map shows that 109 countries are malaria free, 67 are controlling endemic countries, and 32 are malaria-eliminating countries

(Feachem et al., 2010). Recent authoritative reviews provided realistic assessments of the technical, operational and financial feasibilities of malaria elimination (Feachem et al., 2010; Moonen et al., 2010; Sabot et al., 2010; Tatem et al., 2010). Of the 32 countries in the world embarking on elimination, ten are located in the Asian Pacific Region.

To support malaria elimination in Asia Pacific, the Asia Pacific Malaria Elimination Network (APMEN) was founded in 2009 (<http://apmen.org>). It is composed of ten countries (Bhutan, China, Democratic People's Republic of Korea, Indonesia, Malaysia, Philippines, Republic of Korea, the Solomon Islands, Sri Lanka, and Vanuatu) that are pursuing malaria elimination. This network aims to address the unique challenges of malaria elimination in the region through leadership, advocacy, capacity building, knowledge exchange, and building the evidence base. It is apparent that these malaria-eliminating countries all have country-specific characteristics in their malaria situations. For those that border malaria-controlling countries, it is important that measures be implemented to curb malaria reintroduction (Tatem and Smith, 2010). In this aspect, intensified disease surveillance and swift control responses are the basic requirements to prevent the reestablishment of the reintroduced parasites. In some cases, cross-border malaria control, i.e., expansion of malaria control programs from malaria-eliminating countries to neighboring malaria-controlling countries, might be necessary to create buffering zones to thwart reintroduction of the parasite. For example, pilot trials of cross-border malaria control at the Thai-Myanmar and Chinese-Myanmar border regions suggest that organized controls in these regions are feasible (Richards et al., 2009). Obviously, these cross-border activities demand coordination of governments between the neighboring nations.

The NMCPs in the nations of the GMS are mostly organized under the Ministry of Health (MOH) with different organizational structures (WHO, 2008a). For example, the public health system in China is structured mainly through the Centers for Disease Control and Prevention of different municipal levels with coordination of provincial and county health bureaus, whereas the NMCP of Thailand is a division of the Department of Disease Control in the MOH. Depending on the malaria burden and national goal, the NMCPs have received different levels of financial support from their governments. In addition, all countries of the GMS have been supported by the Global Fund to Fight AIDS, Tuberculosis and Malaria (GFATM), which provided the majority of international funding for malaria control in the GMS (WHO, 2008a). The GFATM in China and Thailand focuses on strengthening malaria prevention at the border areas through improved surveillance, reporting, and treatment for cross-border malaria cases in order to respond to local malaria transmission and outbreaks. With an improved malaria situation and better financial support, the NMCPs of GMS nations have revised their national malaria strategies. The following are examples of national malaria control strategies in China and Myanmar, respectively.

The National Malaria Strategy of China for 2010-2015 aims to achieve malaria elimination (Ministry of Health, 2009). Specifically, China aims to 1) achieve annual incidence <math><1/10,000</math> cases/year in 70% of endemic counties and eliminate *P. falciparum* malaria from Hainan Province by 2015; 2) eliminate malaria in 80% of counties currently with an incidence $\geq 1/10,000$ cases/year by 2015; and 3) prevent reintroduction of malaria in places where local transmission has been interrupted. The Revised Malaria Strategy represents a country-wide intensive effort that covers the spectrum from intensified control in endemic areas to prevention of reintroduction in areas without local transmission but being prone to malaria epidemics. The main strategies aim to: 1) ensure access to early, accurate diagnosis, and prompt, effective, safe treatment through public and private sectors; 2) ensure full coverage of the population at risk with appropriate vector control measures; 3) strengthen malaria health education, promotion, and community mobilization efforts and change behavior to maximize utilization of malaria control and elimination services; 4) ensure

comprehensive coverage of vulnerable, poor and marginalized populations at high risk of malaria with appropriate malaria interventions; 5) strengthen the malaria surveillance system by improving case reporting, passive and active case detection, entomological and antimalarial resistance monitoring, and ensuring adequate outbreak response capability; and 6) provide effective program management, based on firm leadership commitment, to enable high quality implementation of strategies from malaria control to elimination (China MOH, 2009).

In August 2010, to meet updated malaria situation and international opportunities, the Myanmar MOH, in collaboration with bilateral and multilateral development partners, national and international non-governmental organizations, developed a new National Strategic Plan for Malaria Prevention and Control (Myanmar MOH, 2010). The goal of the new National Strategic Plan is to reduce malaria morbidity and mortality by at least 50% by 2015 against the baseline level of 2007, and to contribute towards socio-economic development and the Millennium Development Goals. Specifically, the objectives of the new plan are to ensure: 1) at least 80% of the people in high and moderate risk villages in 180 priority townships are protected against malaria by using LLINs complemented with another appropriate vector control methods, where applicable; 2) malaria cases in each township receive quality diagnosis and appropriate treatment in accordance with national guidelines, preferably within 24 h after appearance of symptoms; 3) in 180 priority townships, the communities at risk actively participate in planning and implementing malaria prevention and control interventions; and 4) the Township Health Departments in 180 priority townships are capable of planning, implementing, monitoring and evaluating the malaria prevention and control program with management and technical support from higher levels.

5. Major obstacles and knowledge gaps in malaria control

5.1. Geographical heterogeneity and ‘border malaria’

Malaria control in the GMS is confounded by heterogeneous distribution of malaria, both spatially and amongst populations. At the regional scale, malaria distribution varies greatly among different countries of the GMS. The patchiness is also reflected microgeographically within each country. The majority of malaria endemic regions lie in forests and their peripheries, with the general trend being labeled ‘forest malaria’ (Prothero, 1999). Small villages and communities, with little health infrastructure, are generally more affected by forest malaria than are urban centers. Furthermore, there is a trend for greater infection and malaria-related mortality near international borders, making ‘border malaria’ a concern for malaria prevention (WHO, 2008a). Because of poor accessibility and general negligence, malaria burden in these border areas is the heaviest (Liu et al., 2009; WHO, 2008a).

Border malaria is the result of several socio-cultural, economic, environmental, and political factors. Some border regions are heavily forested, making them an ideal environment for malaria transmission. Along the border regions, many ethnic minorities are concentrated, some of which are politically, economically, culturally, and geographically marginalized from society. They frequently hold occupations or use procurement strategies that increase exposure to malaria vectors (Prothero, 1999). In particular, subsistence activities that are associated with forest areas, such as logging and swidden farming, are likely to increase the risk of infection (Erhart et al., 2005). In addition to poor accessibility, border regions frequently exhibit cultural and linguistic heterogeneity, making health-care education, prevention, and administration complicated. As a result, ethnic minorities and mobile workers on both sides of the border have little access to routine health care services. In recognition of the disproportionate impact of malaria on ethnic minorities, WHO in its “Strengthening Malaria Control for Ethnic Minorities in the Greater Mekong Subregion”

program has identified the Wa, Shan-Lahu-Aka, Karen, Brau-Taliang, Kreung, and Raglai as target minorities for malaria control programs (WHO, 2008b). Another characteristic of border regions is heavy population flow. Yunnan Province of China has more than 4,000 km of border line with Myanmar, Lao PDR, and Vietnam, and population migration across the border lines exceeds 20 million per year (Hu et al., 1998). Motivations for movement vary: some people move to seek better job opportunities, whereas others move to flee from regions afflicted by ethnic tensions, political fighting, wars, or diseases (Martens and Hall, 2000). The mobile and displaced populations are at increased risk of infection as they are exposed to new environments with new vectors and malaria ecologies. Individuals from these groups often fall through the cracks of public health efforts. Studies have shown that populations which reside in conflict zones, such as the Karen, have higher mortality rates regardless of malaria infection (Lee et al., 2006). Beyond an increased risk of infection, migrants may transport malaria parasites to new regions, acting as active transmitters. Reintroduction of malaria is a major challenge for countries transitioning toward malaria elimination where malaria transmission in neighboring countries is intense (Tatem and Smith, 2010). This is particularly the case for China and Thailand, which share lengthy land borders with the highly malaria-endemic Myanmar. For example, in malaria patients over 15 years old in Yunnan, imported cases constituted 22% of total cases in men and 13% in women, and Myanmar was the predominant location of infection for the imported cases (Lin et al., 2009). Since border regions represent the biggest reservoirs for malaria, and frequent malaria introductions by migratory human populations are extremely difficult to monitor, border malaria constitutes one of the biggest obstacles for malaria elimination in these countries. Therefore, 'border malaria' is a shared phenomenon in all the nations of the GMS, requiring in-depth studies and coordinated international efforts.

5.2. Antimalarial drug resistance

The GMS has been an epicenter of antimalarial drug resistance, and parasite strains with resistance to CQ and SP have emerged here and spread to Africa (Anderson and Roper, 2005; Roper et al., 2004). In addition, *P. falciparum* parasites have also developed resistance to other antimalarials such as quinine and mefloquine (Price et al., 2004; Pukrittayakamee et al., 1994). As a result, resistance management for effective control of malaria is of paramount importance. Currently, artemisinins are the only group of antimalarials that are still effective in all the endemic regions, but it is probably a matter of time that artemisinin resistance will develop. The emergence of drug resistance *de novo* is through spontaneous genetic mutations or gene duplications, which are then selected and spread as the result of persistent drug pressure (White, 2004). Many factors in this region favor the development and spread of antimalarial resistance. In field malaria situations, diagnosis of febrile illness relies mostly on symptoms without parasitologic confirmation, and patient adherence to treatment regimens is often poor (Yeung and White, 2005). Despite advocacy for using ACTs, artemisinin monotherapy is still very common, and many manufacturers still ignore the WHO ACT policy (Butler, 2009). The situation is further worsened by circulation of fake and substandard quality drugs (Dondorp et al., 2004; Newton et al., 2008). As a result, parasites are more often exposed to subcurative dosages of the drug, which promotes resistance development. Furthermore, many endemic areas in the GMS are hypoendemic and the resident populations do not develop sufficient levels of immunity to kill parasites that might have survived drug treatments (White and Pongtavornpinyo, 2003). Consequently, it is not surprising that parasites in the region have already shown increased tolerance to artemisinins. In vitro studies in western China have detected reduced susceptibility of *falciparum* parasites to artemisinins (Yang et al., 2003). Historically, artemisinin monotherapy has been associated with a baseline recrudescence rate of 3-5% (Li et al., 1994; Stepniewska et al., 2010); however, this rate has been increased to almost 30% in some regions (WHO, 2010a). Slow parasite clearance has also been reported in western

Thailand (Luxemburger *et al.*, 1998). Three efficacy trials conducted in Cambodia and Thailand have reported reduced efficacy of artesunate-mefloquine on the Thai-Cambodian border, with 15–20% recrudescence rates (Denis *et al.*, 2006; Vijaykadga *et al.*, 2006b). A more recent study in southern Cambodia also reported high failure rates of artesunate-mefloquine therapy, suggesting that this drug combination is beginning to fail in the region and that resistance is not confined to the Thai-Cambodian border (Rogers *et al.*, 2009). Studies from the Thai-Myanmar border have similar findings: 13 years (1995–2007) of continuous artesunate-mefloquine deployment in this area have resulted in a significant decline in efficacy of the three-day regimen of this drug combination (Carrara *et al.*, 2009). To determine whether parasites in the GMS are developing resistance to artemisinins, several recent clinical studies were conducted at the Thai-Cambodian border and in Palin, Cambodia, and they have detected tolerance of the parasites to artesunate, which is manifested as prolonged parasite clearance time (Dondorp *et al.*, 2009; Noedl *et al.*, 2008). These studies provided evidence of an early stage of resistance development in *P. falciparum* to artemisinins. As efforts are being made for the containment of the “resistant” strains, data gathered so far highlight the intrinsic genetic difference among parasite populations and provide a rationale for closer resistance surveillance in “hotspots” of drug resistance (Noedl, 2005). Meanwhile, it is essential that regional efforts and integrated measures be deployed to prevent the spread of MDR, and especially artemisinin-resistant, parasites. WHO has invested significantly in curbing the spread of artemisinin resistance and recently announced a five-point action plan: 1) stop the spread of resistant parasites, 2) increase monitoring and surveillance to evaluate the threat of artemisinin resistance, 3) improve access to diagnostics and rational treatment with ACTs, 4) invest in artemisinin resistance-related research, and 5) motivate action and mobilize resources (WHO, 2011).

5.3. Malaria transmission surveillance and vector control

Accurate identification of vector species is essential to the understanding of vectorial systems, as well as to the design and implementation of vector control methods targeting the main vectors in the area. Mosquito identification is achieved mostly by using morphologic characteristics; however, the presence of sibling species in anopheline mosquitoes requires new techniques for species differentiation. Furthermore, despite the fact that PCR methods have been developed for a number of malaria vector species complexes, methods of molecular diagnosis for understudied species should be developed. Moreover, simple molecular methods for vector identification that can be used in the field settings can greatly aid malaria vector control efforts.

Because vector species have their own unique niche requirements, environmental changes resulting from human activities or global climate change can have major impacts on malaria vector species, potentially causing shifts in vector community structure and malaria transmission dynamics. The impact of environment and climate changes on vector community structure should therefore be examined as they contribute to changing malaria epidemiology. In addition, certain vectors in this region display exophilic biting behaviour, which is responsible for outdoor transmission of malaria. This also raises the issue of vector sampling techniques. Developing cost-effective vector sampling techniques suitable to various epidemiological settings (e.g., forest, swamp, and residential) is critical to malaria vector surveillance and ecological studies.

5.4. Mosquito resistance to insecticides

Within a single year after the introduction of pyrethroids as an insecticide in northern Thailand, resistant mutations of *An. minimus* were recorded (Chareonviriyahp et al., 1999). Since then, *An. minimus* resistance to pyrethroid was reported in Cambodia, Laos, Vietnam, and China (Cui *et al.*, 2006; Prapanthadara *et al.*, 2000; Van Bortel *et al.*, 2008; Yu

et al., 2000). *Anopheles dirus* likely resistant to pyrethroids has been reported in central Vietnam (Van Bortel et al., 2008). High level of resistance in *An. sinensis* was reported in China (Kim et al., 2003). The patchy distribution of pyrethroid resistance in the vector population calls for careful resistance monitoring. Currently, pyrethroid resistance detection is based on the traditional bioassay detection method, *kdr* mutation or detoxification enzyme activities. The bioassay method has several limitations: it requires a large number of field-caught live mosquitoes, which is often difficult to obtain in less abundant sites, and mosquito age and physiological condition confound the bioassay results (WHO, 1998). Standardization of the bioassay method is often difficult across sentinel sites because different sites have different temperatures and humidity. Molecular or biochemical methods are expected to be more sensitive and specific; however, the relative importance of *kdr* mutation or detoxification enzyme in the resistance phenotype is unknown. Understanding the resistance mechanisms is important for the development of molecular or biochemical resistance diagnostic tools, which are critical to the sustained effectiveness of LLINs and IRS program.

5.5. Counterfeit antimalarial drugs

The WHO defines counterfeit/fake drugs as drugs that are “deliberately and fraudulently mislabeled with respect to identity and/or source.” This includes products with the correct or wrong ingredients, without active ingredients, with insufficient active ingredients, or with fake packaging. Substandard drugs refer to genuine drug products that do not meet quality specifications set for them. Counterfeit and poor-quality medicines are a major impediment to the improvement of public health, and are particularly widespread in developing countries (Caudron *et al.*, 2008). The WHO has estimated that about 25% of the medicines consumed in developing countries are counterfeit (WHO, 2006). In many of the developing countries, government agencies such as the Customs and police lack the capability to stop counterfeit drugs entering the countries, and as a result, counterfeit drugs thrive in private markets. Fake drugs not only reduce treatment efficacy and promote resistance development, but also may result in life-threatening complications and even death of the patients. Malaria is a potentially fatal disease, which requires early diagnosis and proper treatment with effective antimalarial drugs. As the progression of malaria from mild to severe disease is rapid, especially in young children, giving drugs that contain little or no active ingredient is parallel to manslaughter (Newton *et al.*, 2006). Counterfeit antimalarial drugs are a huge problem in malaria-endemic countries. Although the situation is slightly better in African countries (Amin and Kokwaro, 2007), Asian countries, especially those in the GMS, face a much greater challenge to combat the growing threat of counterfeit medicine (Dondorp *et al.*, 2004). Even in countries such as Thailand, where legislative procedures are better, substandard antimalarial medicines still constitute a significant proportion of drugs sold. A survey conducted in 2006 on six antimalarials from 27 government hospitals, 27 malaria clinics, and 53 drug stores in Thailand revealed that 15.4% of artesunate, 11.1% of CQ, and 29.4% of quinine were substandard (Vijaykadga *et al.*, 2006a). In other countries of the GMS, the situation is far worse. In Cambodia, a survey conducted in 2006 found that 79% of the antimalarial drugs were not registered and 27% failed the quality tests (Lon *et al.*, 2006). Since 1998, an epidemic of multiple types of counterfeit artesunate tablets has affected malaria patients in the mainland of SE Asia. In this region, between 38% and 53% of artesunate blister packages sampled contained no active ingredients (Dondorp *et al.*, 2004; Hall *et al.*, 2006; Newton *et al.*, 2001, 2003). As many as 14 physical types of fake artesunate have been found (Newton *et al.*, 2006, 2007, 2008). In addition, some counterfeits contained dangerously small amounts of artesunate (Newton *et al.*, 2008). Even some genuine drugs are often substandard (Keoluangkhhot *et al.*, 2008), compromising their expected therapeutic effect. Another problem associated with substandard drugs is expiration and degradation. Because effective treatment of malaria is essential for malaria

control and elimination, monitoring the quality of antimalarial drugs needs to be enforced more effectively. Recently, USAID entered into a new cooperative agreement to expand their joint efforts to combat the proliferation of counterfeit and substandard medicines (<http://www.usp.org/worldwide>), which should greatly strengthen local efforts. In addition, coordination between countries is required to thwart the epidemics of counterfeiting.

Taken together, increased government investment in malaria control in recent years, bolstered by international support such as the GFATM, has led to intensified malaria control efforts and greatly improved the malaria situations in all GMS nations. Moreover, the adoption of malaria elimination as the ultimate goal in certain nations is encouraging. Despite this, the GMS nations are still encountering many obstacles on their way towards eliminating malaria from this entire region. Many of these obstacles including ‘border malaria’, MDR *P. falciparum* parasites, and counterfeit and substandard antimalarial drugs, are shared phenomena of all GMS nations, and require coordinated efforts. In addition, there are important knowledge gaps in malaria epidemiology, vector biology and disease transmission, and mechanisms of drug resistance. Therefore, in-depth research on all these urgent topics is needed to provide timely support for effective malaria control activities so that malaria elimination can ultimately be achieved and sustained in the GMS.

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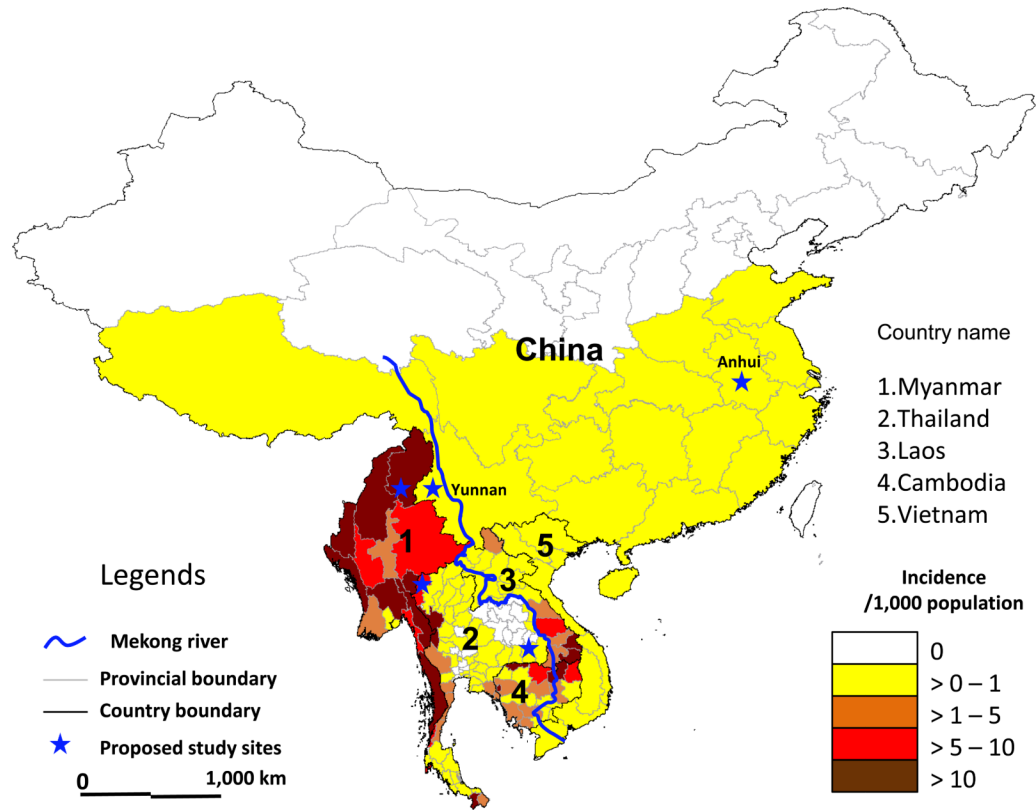


Figure 1. Malaria distribution in the Greater Mekong Subregion.

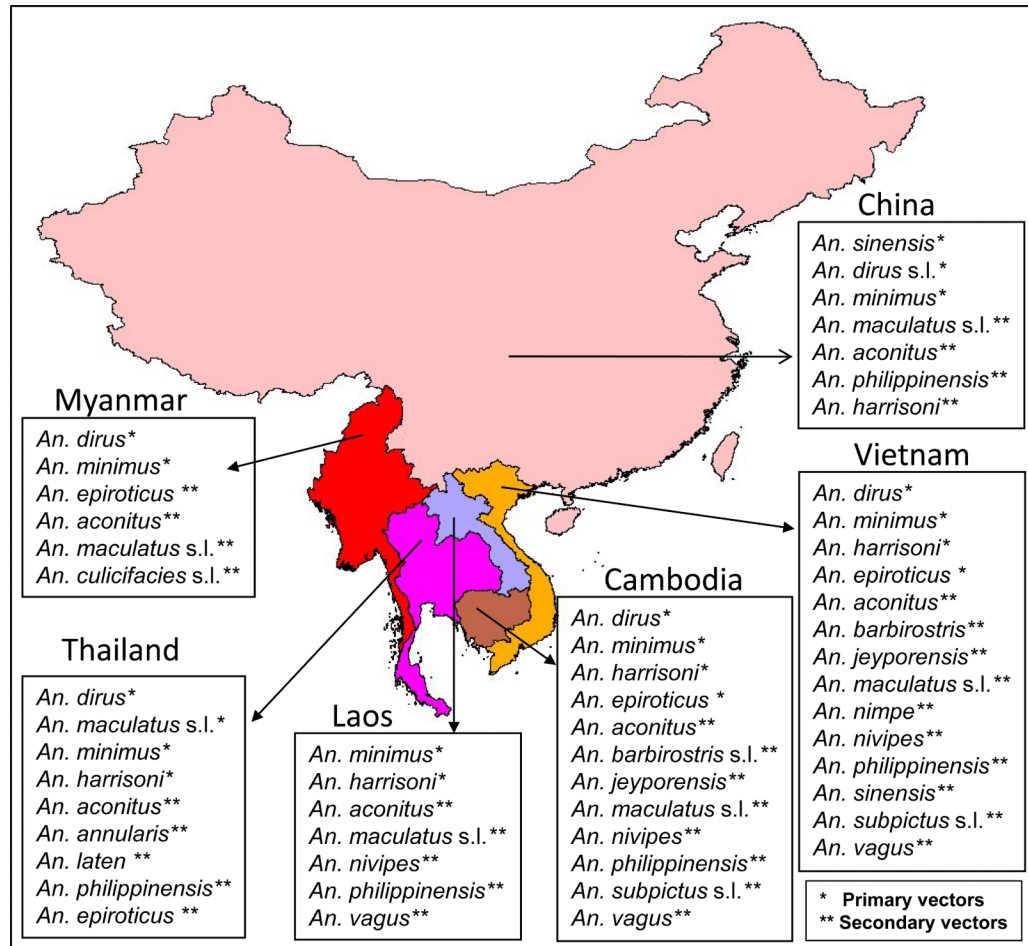


Figure 2. Malaria vector species in the Greater Mekong Subregion (WHO, 2007).

TABLE 1

Country-wide malaria incidence in nations of the Greater Mekong Subregion in 2008.

Country	Reported cases		Annual incidence rate/1,000 population		Proportion of <i>P. falciparum</i>	
	1998	2008	1998	2008	1998	2008
Cambodia	58,874	42,124	4.71	2.87	91.37	35.83
China	27,090	16,650	0.02	0.01	11.47	7.34
Laos	39,031	18,740	7.75	2.96	99.37	25.06
Myanmar	104,753	411,494	3.11	10.15	78.02	87.00
Thailand	131,055	25,449	2.96	0.55	52.70	47.00
Vietnam	72,091	11,354	0.95	0.13	76.39	78.40

Note: Data were obtained from the following web pages:

- 1) <http://www.wpro.who.int/sites/myp/data/malaria/>
- 2) <http://www.wpro.who.int/sites/myp/epidemiology/malaria/>
- 3) http://www.searo.who.int/en/Section10/Section21/Section340_4015.htm
- 4) http://www.actmalaria.net/home/epidemiological_profile.php#base
- 5) http://www.who.int/malaria/publications/country_profiles/en/index.html
- 6) http://www.who.int/malaria/publications/country_profiles/2008/en/index.html.

TABLE 2

A summary of DDT and pyrethroid resistance in major malaria vectors in the GMS.

Species	Country	Insecticide	Bioassay resistance classification*	<i>kdr</i> genotyping**	Reference
<i>An. vagus</i>	Cambodia	DDT	R	L1014S	1
		Pyrethroid	PR		1
	Laos	DDT	R	L1014S	1
		Pyrethroid	R		1
	Vietnam	DDT	R	L1014S	1
		Pyrethroid	R		1
<i>An. sinensis</i>	Vietnam	DDT	R	L1014S	1
		Pyrethroid	R		1
	China	DDT	R	-	2
		Pyrethroid	R	-	2
<i>An. minimus s.l.</i>	Cambodia	DDT	S	No <i>kdr</i> mutation	3, 4
		Pyrethroid	S		3, 4
	Laos	DDT	S	No <i>kdr</i> mutation	3, 4
		Pyrethroid	S		3, 4
	Vietnam	DDT	S	No <i>kdr</i> mutation	3, 4
		Pyrethroid	R		3, 4
	Thailand	DDT	S	No <i>kdr</i> mutation	3, 4
		Pyrethroid	S		3, 4
	China	DDT	PR	-	2
		Pyrethroid	R	-	2
<i>An. lesteri</i>	China	DDT	PR	-	2
		Pyrethroid	PR	-	2
<i>An. dirus s.l.</i>	Cambodia	DDT	S	No <i>kdr</i> mutation	3, 4
		Pyrethroid	S		3, 4
	Laos	DDT	S	No <i>kdr</i> mutation	3, 4
		Pyrethroid	S		3, 4
	Vietnam	DDT	S	No <i>kdr</i> mutation	3, 4
		Pyrethroid	S		3, 4

Species	Country	Insecticide	Bioassay resistance classification*	<i>kdr</i> genotyping**	Reference
<i>An. epiroiticus</i>	Thailand	DDT	S	No <i>kdr</i> mutation	3, 4
		Pyrethroid	S		3, 4
	Cambodia	DDT	PR	No <i>kdr</i> mutation	3, 4
		Pyrethroid	PR		3, 4
	Vietnam	DDT	PR	No <i>kdr</i> mutation	3, 4
		Pyrethroid	R		3, 4
	Thailand	DDT	S	No <i>kdr</i> mutation	3, 4
		Pyrethroid	S		3, 4

References: 1, Verhaeghen et al., 2010; 2, Cui et al., 2006; 3, van Bortel et al., 2008; 4, Verhaeghen et al., 2009

* Bioassay resistance classification is based on WHO (1998): "R", or resistant (mortality<80%), "S", or susceptible (mortality>98%), and "PR", or possible resistance (mortality between 80-97%) after 60 min of exposure to the discriminate dose of the perspective insecticides.

** "-", indicates *kdr* genotyping was not conducted