A CRITICAL REVIEW OF INSECTICIDE RESISTANCE IN US *AEDES ALBOPICTUS*: RESISTANCE STATUS, UNDERLYING MECHANISMS, AND DIRECTIONS FOR FUTURE RESEARCH

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ABSTRACT

Aedes albopictus is a primary or secondary disease vector in Asia that invaded the United States around 1980. It is now present in more than half of US states and continues to expand in range. The willingness to bite in the daytime and the ability to colonize makes this species a target of control operations both to prevent nuisance biting and for public health reasons. As with other species, effective long-term control requires an integrated management strategy and information about efficacy of operational interventions. Studies from Asia, where this species is a primary vector, show that insecticide resistance is a developing concern that can compromise effective control. In this review, we summarize the status of insecticide resistance in US populations of *Ae. albopictus*, examine the current understanding of the mechanisms underlying resistance, and offer suggestions for future research directions.

Key Words: *Aedes albopictus*, insecticide resistance, knockdown resistance, enzymatic resistance

Importance of *Aedes albopictus* as a vector and the need for effective control

Aedes albopictus (Skuse) is one of the most invasive organisms in the world and is now present on all continents except for Antarctica. After several independent introductions into the Americas from the 1940's to early 1980's, established populations of *Ae. albopictus* were finally detected in Texas in 1985 and spread rapidly both north and south (Pratt et al. 1946; Sprenger & Wuithiranyagool 1986; Gratz 2004). Current studies indicate that this spread has reached Canada and more than half the countries in South America (including Argentina in the southern end of the continent), and this increase in range is expected to continue (Peach & Matthews 2022; Kamal et al. 2022; Giordano et al. 2020; Garcia-Rejon et al. 2021).

While this species is often considered a secondary or maintenance vector of dengue, there is evidence to warrant consideration as a primary vector. Previous outbreaks in areas where the primary disease vector, usually *Aedes aegypti* (L.), is not present (including the Yap Islands, Japan and inland regions of China) show that *Ae. albopictus* can effectively transmit disease to humans (Paupy et al. 2009; Gratz 2004). Further, laboratory studies have shown a high level of vectorial competence and the ability to transmit more than 30 viruses (reviewed in: Bonizzoni et al. 2009; Periera-dos-Santos et al. 2020). This becomes more concerning due to the possibility of a rapid change in vectorial capacity by adaptation of a pathogen to a host (Paupy et al. 2009). These threats require that vector control agencies be prepared to manage this species.

Implementation of effective integrated vector management (IVM) is the most effective strategy for long-term management of mosquito vector species (CDC 2023; WHO 2012). One critical element of any IVM program is surveillance both to define the distribution of a species and to determine the presence or absence of insecticide resistance (IR). Most studies of IR have been conducted in Asia due to the continent's long history of *Ae. albopictus*, but a growing body of testing has been conducted in the US.

Insecticide resistance in worldwide *Aedes albopictus* populations

Numerous studies have examined IR in populations of *Ae. albopictus* in its native range in Asia as well as the locations it has invaded (Vontas et al. 2012; Zulfa et al. 2022; Jangir & Prasad 2022; Smith et al. 2016; Moyes et al. 2017; Cui et al. 2006). Likely due to the recent introduction and little testing that had been conducted, the review of Brown (1986) did not note any US populations with IR although many other populations around the world were resistant. Most phenotypic and mechanistic investigations have been conducted in China, where pyrethroid resistance is present in some areas and absent in others (Yiguan et al. 2017; Su et al. 2019; Gan et al. 2021). Larval resistance to pyrethroids generally appears to be more intense than resistance in adults (Yiguan et al. 2017). Very resistant larval populations do exist, and permethrin resistance ratios of up to 80-fold have been found in larval populations from Huangpu and Nansha. The Huangpu population was also broadly resistant to pyrethroids as a class with nearly 200-fold resistance to deltamethrin and nearly 300-fold resistance to cypermethrin but had little resistance to organophosphates (OPs). In these same populations, adult resistance to permethrin was much less intense with approximately 75% mortality in the Huangpu and Nansha populations at the diagnostic time. Clearly there is local variation in IR across populations; a similar study from Guangzhou examining four populations identified adults resistant to permethrin but susceptible to malathion while the larvae were resistant to the OP temephos (Su et al. 2019).

This pattern observed in China is common in other areas in Asia and Africa. In Laos, permethrin susceptibility was seen in adults of all tested populations, except for one with marginal resistance, while the same populations had a range of resistance to malathion (Tangena et al. 2018). Other studies identified the same pattern with a mix of susceptible and resistant populations in Thailand, Malaysia, India, and Cameroon, although none approach the intensity of the resistance seen in Guangzhou (Singh et al. 2013; Nurul-Nastasea et al. 2023; Sumitha et al. 2023; Yougang et al. 2020; Chareonviriyaphap et al. 2013). The existing body of research spanning various regions of Asia highlights the heterogeneous nature of insecticide resistance in *Ae. albopictus* populations, emphasizing the need for continued monitoring and tailored management strategies in the region.

Insecticide resistance research on US populations of *Ae. albopictus* is less developed since it has not been a primary focus of vector control and has a relatively short history of invasion. However, studies assessing IR in US populations are becoming more common, and now populations from at least 20 US states have been studied using a variety of assays, active ingredients, and life stages (Figure 1 & Table 1). Over 40 Florida populations have been examined (Wesson 1990; Liu et al. 2004; Alimi et al. 2013; Marcombe et al. 2014; Xu et al. 2016; Waits et al. 2017; Richards et al. 2017, 2018; Estep et al. 2018; Parker et al. 2020; Jiang 2022). Most of these populations were tested by CDC bottle bioassay or WHO tube assays and were susceptible to permethrin though several populations

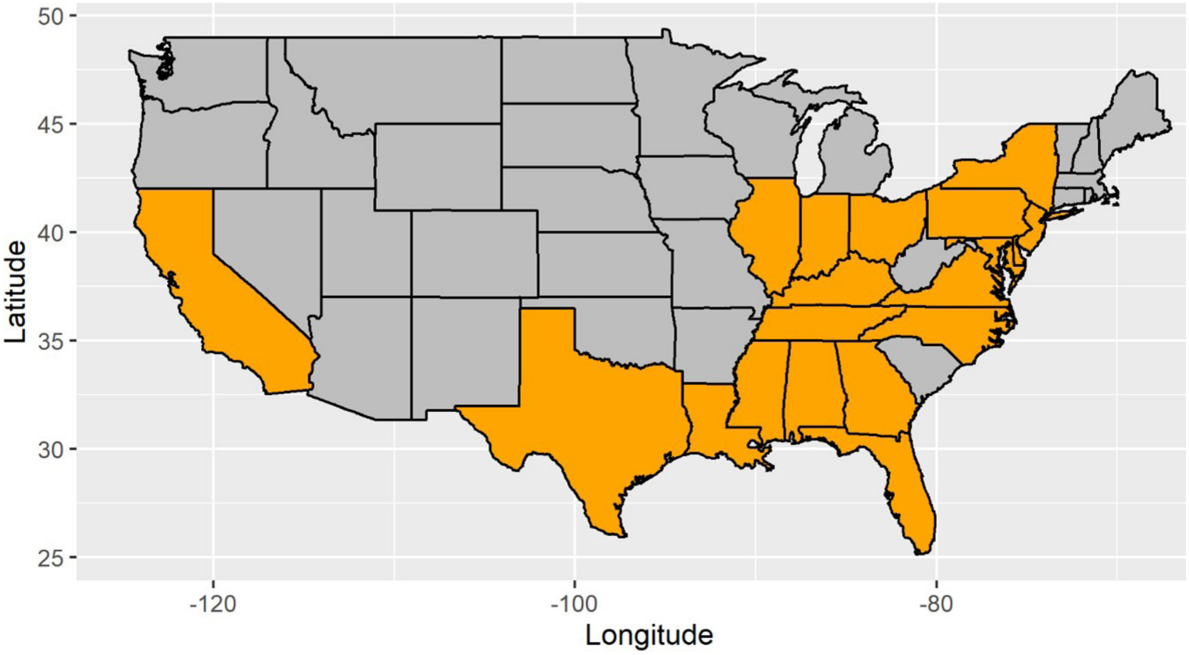

Figure 1. Location of states with at least one population of *Aedes albopictus* tested for insecticide resistance. Hawaii not shown. See Table 1 for specific studies. Map produced in R (R Core Team 2018).

Table 1. Summary of published studies that have examined insecticide resistance in US *Aedes albopictus*.

Publication Year	State	Adult[a] (test method – Active Ingredient result)	Larval[a] (test method – Active Ingredient result)	Mechanism	Reference
1988	TX	Topical Assay – MAL 4/4 RES; Scourge 4/4 SUS [b]			Khoo et al. 1988
1989	KY		Modified WHO Assay – PER 1/1 RR<2; MAL 1/1 RR<2; *Bti* 1/1 RR<2		Cilek et al. 1989
1989	TX	Plapp Assay – SPs 2/2 RR~2; malathion 2/2 RR<3			Robert & Olson 1989
1990	IN, IL, FL, KY, OH, TX, HI		Modified WHO Assay – temephos 10/13 SUS, 3/13 DR; chlorpyrifos 10/13 SUS, 3/13 DR; malathion 10/13 SUS, 3/13 DR		Wesson 1990
1993	MD	WHO Assay – MAL 2/2 SUS	WHO Assay – temephos 2/2 SUS		Sweeney 1993
1996	TX	Plapp Assay – PER 1/1 RR~1; chlorpyrifos 1/1 RR~1; MAL 1/1 RR<6			Sames et al. 1996
2003	CA	Multiple populations eradicated using formulated pyrethroids			Linthicum et al. 2003
2004	AL, FL		Larval Assay – PER 4/4 RR<5; deltamethrin 2/4 RR<5, 1/4 5<RR<10, 1/4 RR=22; chlorpyrifos 4/4 RR>9; malathion 4/4 RR<3; *Bti* RR<4		Liu et al. 2004
2013	FL	Bottle Assay – permethrin 1/1 SUS			Alimi et al. 2013
2014	NJ, PA, FL	WHO Assay – deltamethrin 7/7 RR<2; DDT 7/7 RR<2; MAL 5/7 RR<2, 2/7 2<RR<3	WHO Assay – temephos 8/8 RR<1.5; *Bti* 8/8 RR<1.8	1 population with 1534L, no clear enzymatic pattern	Marcombe et al. 2014
2016	CA, FL, HI, TX			3/4 no *kdr*, 1/4 12% 1534S	Xu et al. 2016
2016	GA	Modified Topical – Talstar 1/1 SUS; suspend 1/1 SUS			Nguyen 2016
2017	FL	Topical Assay – PER 3/3 RR<2.5; CDC Assay – MAL 1/1 SUS			Waits et al. 2017
2017	CA, VA, TN, FL, LA, AL, GA, TX	CDC Assay – PER 8/12 SUS, 4/12 DR; MAL 2/12 DR, 10/12 RES			Richards et al. 2017
2018	TX, FL, LA, CA	Modified CDC Assay – PER 7/8 SUS, 1/8 DR; MAL 5/8, 1/8 DR, 1/8 RES			Richards et al. 2018
2018	IL	Modified CDC Assay – PER 6/6 minimal resistance; MAL 6/6 minimal resistance			Kim & Stone 2018
2018	FL	Topical Assay – PER 6/6 RR<2.5			Estep et al. 2018

2019	NC	CDC Assay – SPs 8/8 SUS: low level to MAL			Richards et al. 2019
2019	MS	CDC Assay – PER 16/16 SUS; MAL 5/18 SUS, 8/18 DR, 5/18 RES			McInnis et al. 2019
2020	FL	CDC – PER 11/35 SUS, 7/35 DR, 19/35 RES; MAL 6/38 SUS, 12/38 DR, 20/38 RES			Parker et al. 2020
2021	DE, NY, NJ	CDC Assay – PER 2/2 "low"; sumithrin 2/3 "none", 1/3 "low"; etofenprox 1/2 "none", 1/2 "high"	WHO Assay – methoprene 3/3 SUS; *Bti* 3/3 SUS		Burtis et al. 2021
2021	TX	CDC Assay – SP 1/1 SUS			Salinas et al. 2021
2022	NC			11/13 no *kdr*, 1/13 <5% 1534S, 1/13 <25% 1534S	Abernathy et al. 2022
2022	FL	CDC Assay – PER 3/3 SUS at DT; MAL 3/3 SUS at DT			Jiang 2022
2022	AL	CDC Assay – permethrin (15µg/bottle) 6/6 SUS; malathion (50µg/bottle) 5/6 SUS, 1/6 RES	WHO Assay – permethrin 6/6 SUS; malathion 5/6 SUS, 1/6 RES		Wang et al. 2022
2023	TX	Topical Assay – PER 4/4, RR<3			Estep et al. 2023

[a] Abbreviations used: SP = synthetic pyrethroid, OP = organophosphate, CDC = Centers for Disease Control, WHO = World Health Organization, SUS = susceptible, DR = developing resistance, RES = resistant, RR = resistance ratio, DT = diagnostic time, MAL = malathion, PER = permethrin, *Bti = Bacillus thuringensis Israelensis.*

[b] Notation for results of testing: MAL 4/4 RES; Scourge 4/4 SUS = 4 of 4 populations RES to malathion and 4 of 4 populations SUS to Scourge.

had some levels of resistance. Topical application studies confirm low levels of pyrethroid resistance at less than 3-fold. Six studies noted some resistance in adults to malathion, but no studies have quantified this by topical application. Larval bioassay studies have identified several Florida populations with some (<5-fold) resistance to chlorpyrifos and malathion.

Several other statewide studies show this same pattern. Resistance in Texas *Ae. albopictus* populations has been examined over several decades. In two of three studies conducted within a decade of the initial invasion, resistance to malathion was present (Khoo et al. 1988; Robert & Olson 1989; Sames et al. 1996). Notably, no reduction in malathion resistance was observed even after 17 generations in the laboratory, leading to the conclusion that whatever factor was responsible for the IR was of minimal fitness cost as it did not decrease in the absence of any insecticide pressuring (Khoo et al. 1988). Recent studies have not found strong IR to pyrethroids even though *Ae. aegypti* collected from the same locations were resistant (Salinas et al. 2021; Estep et al. 2023). In Mississippi, 16 populations were susceptible to permethrin, five were susceptible to malathion, and 13 had some level of malathion IR (McInnis et al. 2019). In state specific studies from North Carolina, Alabama, and Illinois, all populations tested were susceptible to permethrin (Richards et al. 2019; Wang et al. 2022; Kim & Stone 2018). Broader area-wide studies also show the same pattern of relatively low permethrin IR (Marcombe et al. 2014; Xu et al. 2016; Richards et al. 2017, 2018; Burtis et al. 2021). Several of these same studies have found that malathion resistance is present, again at relatively low intensity in *Ae. albopictus* populations. Only a few studies have examined IR in US larval *Ae. albopictus* and appear to agree with reports from Asia that while IR is often low, in specific populations larval IR can be much higher than in adults (Cilek et al. 1989; Wesson 1990; Sweeney 1993; Liu et al. 2004; Marcombe et al. 2014; Burtis et al. 2021; Wang et al. 2022).

While some resistance to synthetic pyrethroids (SPs) and OPs has been detected in laboratory assays, it is not clear whether this IR correlates with reduced efficacy for operational sprays with formulated products. An early study from Texas did find low levels of IR to formulated malathion but none to a formulated SP, while a second study only found susceptible populations (Khoo et al. 1988; Robert & Olson 1989). Susceptibility to SP formulations in Florida, susceptibility to formulated products in Georgia, and the temporary eradication in California using synergized SPs all indicate that the IR observed in laboratories may not yet be above the threshold needed to reduce field efficacy (Alimi et al. 2013; Nguyen 2016; Linthicum et al. 2001).

Mechanistic basis of insecticide resistance in *Aedes albopictus*

Phenotypic resistance to a given active ingredient (AI) is the result of the individual contribution of multiple mechanisms (Liu 2015; Liu et al. 2006). The demonstrated mechanisms that result in IR in mosquitoes are heritable target site changes that reduce the efficacy of pesticides, enhanced enzymatic activity from a variety of esterases, cytochrome P450s, glutathione-S-transferases and transport proteins that degrade or remove the AI from the site of action, changes in cuticular penetration that reduce the contact of the AI with the target or changes in behavior that result in less contact with the AI (Liu 2015; Sparks et al 1989; Siddiqui et al 2023). In *Aedes* mosquitoes, the major contributors to IR are target site mutations and enzymatic resistance (Gan et al. 2021; Ranson et al. 2010; Vontas et al. 2012). While some exploration of these mechanisms in *Ae. albopictus* has been conducted, it is an area of active research and is currently much less developed than the mechanistic studies conducted in *Ae. aegypti* and *Culex quinquefasciatus* Say (Smith et al. 2016; Scott et al. 2015). How these mechanisms contribute, and the relative importance of the various mechanisms to the overall phenotype, is a rapidly developing area of research. Initial studies indicate that both knockdown resistance (*kdr*) mutations in the voltage gated sodium channel (VGSC) and enzymatic resistance play a role in *Ae. albopictus* but with unique elements compared to the more widely explored vectors.

Knockdown resistance in *Aedes albopictus*

Most of the research to identify and define *kdr* mutations in *Ae. albopictus* has been conducted in Asia, where the species has a long history and demonstrated ability to serve as a primary vector of disease. Mutations at positions 1532, 1534, and 1016 in the VGSC have been discovered in some *Ae. albopictus* populations and appear to be widely distributed. Mutations at 1534, initially reported as the substitution of the normal phenylalanine (F) with a cysteine (C) (1534F→1534C), and subsequently also as lysine (L) or serine (S), have been described (Kasai et al. 2011; Marcombe et al. 2014; Chen et al. 2016; Xu et al. 2016; Gao et al. 2018). Studies are mixed on the importance of these 1534 substitutions, but these mutations have been generally linked to increased resistance (Chen et al. 2016). Several associated studies have attempted to parse the effect of these *kdr* mutations based on post-hoc analysis of assay data and have associated 1534S with increasing permethrin resistance. They found that 1534S and 1534L were overrepresented in the portion of the population that survived exposure during bioassays. In contrast, 1534C was not associated with the resistant fraction (Gao et al. 2018; Su et al. 2019; Li et al. 2018). Gao et al. (2018) also identified a 1532 isoleucine (I) to threonine (T) (1532I→1532T) mutation that did not correlate with permethrin resistance. More recently, a 1016 valine (V) to glycine (G) (1016V→1016G) mutation has been found in locations in China and Italy (Kasai et al. 2016; Zhou et al. 2019). Just as in *Ae. aegypti*, this 1016G mutation has been unequivocally demonstrated to result in intense resistance to pyrethroids (Kasai et al. 2019). Recent studies have surveyed for the presence of these 1534 and 1016 mutations as well as for potential mutations at 989 and 1011 and found them to be present in some populations in China but the toxicologic impact and operational importance of these mutations is unclear (Chen et al. 2021; Wei et al. 2021; Su et al. 2019; Wu et al. 2021; Zhao et al. 2023).

Within the US, currently only mutations at the 1534 position of the *Ae. albopictus* sodium channel have been identified. The first review of IR in US strains found the presence of 1534L in one population (Marcombe et al. 2014). Both 1534L and 1534S were identified in a 2016 study with 1534S detected in a Florida population (Xu et al. 2016). Twenty-five percent of tested North Carolina populations contained some organisms with 1534L (Abernathy et al. 2022). Findings of few *kdr* mutations in US populations could be simply due to a lack of widespread assessment but may also be explained by the phenotypic resistance data. Current IR testing has not identified strong pyrethroid resistance (when quantified) in US *Ae. albopictus* populations, thus mutations that correlate with pyrethroid resistance should also be uncommon (Khoo et al. 1988; Sames et al. 1996; Nguyen 2016; Waits et al. 2017; Estep et al. 2023).

Enzymatic resistance in *Aedes albopictus*

Based on other mosquito species, enzymatic (metabolic) mechanisms likely play some role in *Ae. albopictus* IR, but the definitive proof for their involvement is rather unclear. Several synergist studies, using piperonyl butoxide, an inhibitor of cytochrome P450 activity that can increase the pesticidal efficacy of a given quantity of AI have been conducted on *Ae. albopictus.* These studies have shown recovery in moderately resistant strains when the synergist piperonyl butoxide (PBO) is used, which points to the involvement of enzymatic activities in the low levels of IR observed (Ishak et al. 2015; Rahman et al. 2021).

Transcriptome studies have identified upregulated transcripts for carboxylesterases, cuticle proteins, glycosyltransferases, and cytochrome P450s (Grigoraki et al. 2015; Ishak et al. 2016). Upregulated cuticle protein transcripts, not normally considered enzymatic resistance, were identified in both studies along with traditional cytochrome P450s and glycosyltransferases. The overexpressed carboxylesterases identified by Grigoraki et al. (2015) were found to be present in two globally disparate strains (Athens, Greece and US) in a follow up study, but no phenotypic resistance was assessed to confirm that resistance was present along with the elevated expression (Grigoraki et al. 2017). The cytochrome P450 CYP6P12 was identified as highly overexpressed in a strain slightly resistant to both permethrin and deltamethrin, but puzzlingly, expression in *Drosophila melanogaster* showed that CYP6P12 expression reduced mortality from deltamethrin exposure while providing no protection from mortality caused by permethrin (Ishak et al. 2016).

Adding to the difficulties in assessing the role of metabolic resistance in *Ae. albopictus* are numerous studies that show susceptible strains with significantly higher enzymatic activities even when resistance is minimal or absent. A strain of *Ae. albopictus* from Haiti had significantly higher levels of alpha esterase and oxidase activity than *Ae. aegypti* even though the *Ae. albopictus* were much more susceptible to pesticide exposure than the *Ae. aegypti* (McAllister et al. 2012). Two strains from the Central African Republic had significantly increased activity for esterases, glutathione-S-transferases, and cytochrome P450s but had 87% and 100% mortality after exposure to deltamethrin in the WHO tube assay. Four other strains that did not have the same pattern of increased enzymatic activity were also susceptible, with 94-100% mortality. All six strains examined were susceptible (⩾ 94% mortality) to representative carbamates and organophosphates. Again, the increased enzyme activity identified in the two strains did not result in phenotypic resistance (Ngoaguoni et al. 2016). Metabolic and phenotypic resistance were examined in eight strains of *Ae. albopictus* in Thailand. All were susceptible to permethrin and deltamethrin (98-100% mortality), susceptible to organophosphate (91-100% mortality), and susceptible to a carbamate (98-100% mortality). Though phenotypic resistance was essentially absent, enzymatic activities varied considerably. These susceptible strains had oxidase and beta-esterase activities that varied nearly 6-fold and alpha-esterase activities that varied almost 4-fold. Glutathione-S-transferase and acetylcholinesterase activities varied by less than 2-fold (Pethuan et al. 2007; Jirakanjanakit et al. 2007). The only US study that examined metabolic resistance also found no particular pattern of overexpression (Marcombe et al. 2014). At this point, the contributions of metabolic resistance are unclear for phenotypic resistance in *Ae. albopictus,* and much work remains to be done before a clear picture emerges. The cautions echoed in Vontas et al. (2020) about the confusing role of cytochrome P450s in *Ae. albopictus* IR may well be applicable to the other families of metabolic enzymes based on the conflicting results between activity and observed resistance; if enzyme levels are high in strains without resistance, it is unlikely to be an indicator of resistance.

Future directions for insecticide resistance research in *Aedes albopictus*

Resistance testing should continue as a standard part of a resistance monitoring program, and baseline information should be generated for communities that do not know the IR status of local populations. Though the latest studies indicate that there is only low-level IR to pyrethroids in US *Ae. albopictus* populations, the recent discovery of the 1016G mutation in Asia and the demonstration that it results in strong pyrethroid resistance makes monitoring important (Kasai et al. 2019). For those US states that already have an IR monitoring baseline, continued testing will show if IR is changing. Just as *Ae. albopictus* originally entered the US through global trade, it is logical to assume that as 1016G spreads in Asia it may also infiltrate the US by the same method. If the spread of this strong IR allele is analogous to the spread of the ensemble 1016I/1534C mutations in *Ae. aegypti*, rapid loss of susceptibility can occur. Monitoring IR using standard methods is critical for early detection (Baltzegar et al. 2021).

New research tools also need to be developed to assist in future IR research efforts. One or more standard *Ae. albopictus* colonies need to be developed. This includes a few susceptible strains that can become widely available lab standards like the Rockefeller, Liverpool, Orlando, New Orleans, and Bora-Bora strains have for *Ae. aegypti.* Several susceptible *Ae. albopictus* strains were used in the studies cited in this review, but we know of no study that has compared them to determine if they have similar toxicologic baselines. In addition to susceptible strains, the development and characterization of IR strains analogous to the pyrethroid resistant Puerto Rico strain of *Ae. aegypti* available from BEI Resources/NIAID would also be valuable (BEIResources, 2024). Since *Ae. albopictus* appears to have some level of organophosphate resistance in addition to pyrethroid resistance, a strain with this phenotype would be a crucial tool for use by researchers.

Additional molecular tools would also be valuable, such as a protocol or kit of reagents to allow for the rapid assessment of the presence of the specific *Ae. albopictus* 1016G mutation. A simple melt curve assay primer set would vastly increase the ability to identify the spread of this IR allele. Considering that OP resistance has been observed and is not well explained by the extant enzymatic data, assessment of the presence of characteristic AChE mutations is a critical need. If identified, they should be functionally verified and rapid assessment tools should be designed (Weill et al. 2004; Kasai et al. 2019).

More research needs to be conducted to conclusively validate the role of enzymatic resistance as Kasai et al. (2019) have done for the 1016G *kdr* mutation. As mentioned above, numerous studies found significantly higher enzymatic activity in strains that had little or no IR which calls into question the organismal level importance of these laboratory findings. If IR is absent but transcript or enzyme levels are high, it is unlikely that the finding is important as a marker of resistance. Notably, several authors that note these higher activity levels also raise this same question. Much more work is needed to define the importance of these factors on phenotypic IR.

Another need for effective control of this species is to resolve the role that the differing physiology of the various life stages play in IR. We currently lack much understanding of the differences in IR between aquatic and terrestrial forms as few studies have examined both. The extant data appears to show that larval IR can be more intense than in adults, but since mechanistic studies of differing life stages are limited, the assumption inherent in translating results from larvae to adults or vice versa on the IR phenotype is tenuous.

CONCLUSION

Aedes albopictus is an important vector in parts of the world and is therefore an important target of control. It is also clear that *Ae. albopictus* often tends to have resistance to organophosphates in the immature stages. With the recent demonstration and validation of strong, target site-based resistance to synthetic pyrethroids in adults in Asia, concern over the wider spread of strong IR is warranted. As of today, we have not observed strong IR in adult US populations. However, the spread of strong IR in *Ae. aegypti* was extremely rapid and should serve as a warning to continue regular surveillance and be prepared with the tools and methods to define and respond effectively to strong IR in *Ae. albopictus* when it arrives.

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