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A review: mosquito proteomics and its potential role in insecticide resistance detection

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Abstract
Vector control remains the best technique to fight mosquito-borne infections worldwide, and it is an essential entity of a global strategy for the management of mosquito-borne diseases. Insecticide continues to be the widely used method to achieve vector eradication and elimination. However, the increased use of insecticide has contributed to the establishment of resistance, jeopardizing the effectiveness of control methods globally. The outcome of the resistance is the enormous human and economic loss. The use of mosquito proteome analyses to identify the expression of the whole complement of proteins in a cell or organism at a particular moment, and in response to various physiological changes and stress, has indeed revealed so much of protein behaviors. This paper reviews the scenario and mechanisms of insecticide resistance in mosquitoes and also highlighted proteins associated with insecticide resistance compared to susceptible mosquito strains under different physiological states. Differentially expressed proteins hold the key in elucidating resistant patterns and possible development of biomarkers in predicting resistance in field strain mosquitoes as well as surveillance for control strategies.

Keywords: Mosquito-borne diseases, insecticide resistance, proteomics, differential protein expression

1. Introduction

Arthropod like mosquitoes, reduviid bugs, blackflies, tsetse flies, sand flies, lice, and ticks transmit deadly pathogens and parasites and cause myriad of human diseases. Arthropod-borne infections continued to be a significant public health problem in tropical and subtropical regions. Despite several decades of vector control efforts, mosquitoes are considered the most threatening of the arthropod in global public health [1, 2]. Malaria caused by *Anopheles* still causes significant morbidity and mortality with an estimated 228 million cases worldwide and divergently affect populations in Sub-Saharan Africa (93%) followed by South-Eastern Asia (3.4%) and the Eastern Mediterranean Countries (2.1%) [3]. Arthropod-borne viruses such as dengue, chikungunya, yellow fever and Zika add significantly to the global burden of vector-borne diseases [1]. More than 5 billion people worldwide live in areas of possible transmission. An estimated total number of 4 billion people are at risk of contracting dengue virus alone because they live in areas overwhelmed by the vector [4].

The principal method available for controlling and preventing many vector-borne diseases still vector control measures. There is an authorized vaccine against dengue virus infection but not widely used because of safety issues. Malaria RTS, S vaccine is not 100% effective either, though promising with 36% efficacy rate, for the time being, vector control is the only means to protect the public against chikungunya, Zika, and West Nile diseases [5]. Vector control acts to reduce the risk of pathogen transmission by destroying vector populations and reducing their contact with a human. Insecticide based vector control techniques work by eradicating immature mosquito stages using compounds like temephos (larvicide) with very low mammalian toxicity. Indoor residual spraying (IRS), peridomestic space spraying targeted adult stage vectors, and or minimizing vector-human contact through topical repellent, house screening, insecticide-treated bed nets (ITNs) and insecticide-treated dog collars [1].

Insecticides have long been a critical entity of global vector control strategies to manage mosquito-borne diseases, and pyrethroids are mainly used worldwide for indoor spraying because of their safety and efficiency [6]. Over the years, insecticides have significantly reduced the burden of such diseases, from malaria control programs in Sub-Saharan Africa, dengue vector control in South-East Asia, as well as environmental and vector control strategies in South America against *Aedes* and other vectors. This decline in disease burden is threatened by insecticide resistance development and capable of jeopardizing global health policies, thereby reversing the successes recorded.

Perhaps vector control measures are the primary means for controlling and eradicating mosquito-borne diseases in resource-constrained countries [1]. The use of pyrethroid treated bed nets in Africa is a contributing factor in spreading resistance against malaria vector and other vector-borne diseases [7].
Nevertheless, the mosquitoes have already become resistant due to prolong use of insecticides. The use of a particular class of insecticide would no longer be an effective option to control vector-borne diseases. The use of insecticides for decades has prompted selective pressures that minimized the efficacy of the ongoing vector control measures. The failure in vector control will translate to the resurgence of vector-borne disease, and the overall impact on public health would be enormous [8]. To further understand the molecular mechanism involved in mosquito insecticides resistance, a few studies have embarked on investigating the proteome of resistant compared to susceptible mosquitoes to gain critical insight. Therefore, this review highlights the use of proteomics technology, to unravel not only the proteome of mosquito and its component but also the resistant mosquito strains. The outcome perhaps could be utilized for the possible development of biological markers in predicting resistance in the field strain mosquitoes as well as surveillance for control strategies.

2. Pyrethroids

Pyrethroids are a group of synthetic pyrethrin insecticides. Pyrethrins are esters of cyclopropane carboxylic acid, present mainly in *Chrysanthemum* flowers. The chemical configurations of pyrethroid share similarities across the class, and the basic acid/alcohol moiety are conserved [9].
Pyrethroids consist of 6 esters in the non-polar extract solvent of the flower head and occur in nature to produce the pyrethrum extract.
Pyrethrum is perhaps the most potent plant-derived insecticide with immense insecticidal properties and low toxicity to mammalian cells. Pyrethroid insecticides are neurotoxins where they alter the usual action in the insect nerves, disrupting the voltage-sensitive Na^+ channel gene by depolarizing the neuronal pathways, thereby paralyzing and rendering the insect dead [10, 11].

2.1 Resistance mechanisms: pyrethroid

Resistance in mosquito vectors is either physiological resistance or behavioral evasion against pyrethroids. Physiological resistance is the survival of mosquitoes and other vectors subjected to a chemical-based insecticide that generally causes insect mortality. These include target sites insensitivity in the voltage-gated Na^+ channel and insecticide detoxification. Behavioral evasion refers to any change in the routine of a vector, to minimize contact with harmful compounds or flee the deadly outcome of an insecticide [11, 12].

2.1.1 Knockdown resistance

Pyrethroids deliver their effects mainly through binding to the Na+ channel, modifying its gating features, and allowing it in an open position for a longer duration. Changes in the Na+ channel structure in either point mutation or substitution from single nucleotide polymorphisms lead to insensitivity to insecticides in the Na^+ channels of the mosquito nervous system through the removal of the binding affinity of the insecticides to proteins, and it blocks the formation of the electric current flow via the membrane. The pause in the electrical current contributes to the immediate immobilization and death of the insect [13]. The term knockdown resistance (*kdr*) is to denote cases of insensitivity to insecticides in mosquitoes because of the target site modification in the Na^+ channels [6, 14].

2.1.2 Metabolic detoxification/resistance

Metabolic detoxification in the mosquito vectors includes alteration of the expression sequence in some of the complex enzyme group. The modification results in an increased rate of detoxification process of pyrethroid. Cytochrome P450 monooxygenases, esterases, and glutathione transferases GSTs rise the biodegradation of insecticides in this mechanism [15]. The enzyme family cytochrome P450 is mainly responsible for insecticide insensitivity in mosquitoes. P450 enzymes can efficiently metabolize drugs, plant-based toxins, and chemical insecticides. Another essential property of P450 was the overexpression in insecticides-resistant mosquitoes and are associated with the development of resistant [11]. The carboxy/choline esterases genes are made up of α and or β hydrolase fold. These genes contain active catalytically enzymes and non-catalytic enzymes in the neuron signaling process. Mosquitoes typically have fewer GSTs, though alternative splicing of couple GST mosquito genes raises their percentage via transcription [11].
Insecticide insensitivity is also related to cuticle hardening and thickening by reducing the uptake of the insecticides' harmful effects from reaching the target site. The harder cuticle has shown to be abundant in resistant mosquito strain than in susceptible mosquito strain [16]. Perhaps this is due to the adaptation of the feeding and resting pattern of mosquitoes to minimize contact or flee from insecticides. Contact irritancy occurs when mosquitoes turn away from insecticide-exposed sites upon exposure to the harmful compound. Meanwhile, non-contact spatial repellent induces mosquitoes to avoid contact with the deadly compounds. The continued pyrethroid usage stimulated an array of irritancy, resulting in mosquitoes surviving in insecticide coated surfaces [17]. Table 1 shows evidence of insecticide distribution detected in certain countries and their mechanisms.

Table 1: Mutation in the sodium channel proteins associated with pyrethroid resistance in mosquito vectors and their corresponding resistance mechanism.

Country	Species	Mutation	Mechanism	Method	Ref
East Africa	*An. Gambiae*	L1014S	*Kdr*	PCR	[18]
Asia Latin America Africa	*Ae. aegypti*	L75TW, I104M, G16V, V109G	*Kdr*/Metabolic resistance	RT-PCR	[19]
UAE	*An. stephensi*	L31F	*Kdr*	PCR	[20]
USA	*Cx. quinquefasciatus*	L1014, L1014S	*Kdr*/P450 monooxygenase mediated metabolism	PCR	[21]
Latin America	*Ae. aegypti*	Iso1016, Val1016	*Kdr*	Allele-specific PCR	[22]

Taiwan	*Ae. aegypti*	D1794Y	*Kdr*	RT PCR	[23]
Thailand	*Ae. aegypti*	F1552C	*Kdr*	PCR	[24]
USA	*Cx.quinquefasciatus*	CYP6AA7, CYP9J34, CYP9M10	Metabolic resistance	qRT-PCR	[25]
Malaysia	*Ae. aegypti*	F1534C, V1016G	*Kdr*	Allele-specific PCR	[12]
India	*Ae. aegypti*	F1534C, T1520I	*Kdr*	Allele-specific PCR	[26]
Malaysia	*Ae. albopictus*	CYP6P12	Metabolic resistance	Transcription analysis	[27]
Malaysia	*Ae. aegypti*	CYP9J27, CYP9J26, CYP9M4	Metabolic Resistance	Transcription analysis	[14]
Sri Lanka	*Ae. aegypti*	F1534C, V1016G, S989P	*Kdr*	PCR	[13]
USA	*Ae. aegypti*	CYP9J28, CYPJ10	Metabolic resistance	Transcription analysis, PCR	[28]
Central Africa	*An. coluzzi*	L1014F	*Kdr*	Taqman genotyping assay	[29]
Southern Africa	*An. funestus*	CYP6P9a	Metabolic resistance	qRT-PCR	[16]
Laos	*Ae. aegypti*	V1016G, F1534C, CYP6BB2, CYP6P12	*Kdr*/Metabolic resistance	qPCR	[8]

3. Proteomics analyses of mosquitoes

Proteomics is comparatively a new field in molecular medicine. It entails the analysis of complete proteins in cells, tissues, organ systems or the organism. Mosquito proteomics was revealed in 2002 when the *Anopheles* mosquito's preliminary genome was published. Genomics alone cannot fully explain the biochemistry of protein. Proteomics study the protein multisubunit, abundance, complexity, half-life and post-translational modifications. Quantitative proteomics is often the approach used in a resistance study. Thus, full comparative analyses of specific proteins would be performed during certain biological processes or even during exposure to a particular treatment [30]. The comparison extensively reveals specific differential protein expression and distinct peptides that could be associated with specific biological functions and properties.

In mosquito proteomics studies, a bottom-up investigation is the choice of method. It consists of analyses from peptides to proteins of complex cells and tissues made up of several proteins at varying ratios. In the bottom-up approach, to breakdown proteins in the sample into fragment peptides, proteolytic enzymes are applied. The peptides are analyzed using mass spectrometry (MS/MS) such as LC-MS/MS and MALDI-TOF/TOF. The peptides are ionized by electrospray ionization system (ESI) or matrix laser-assisted deionization (MALDI) into parent ions. Then, parent ions are selected and fragmented into daughter ions in the collision-induced dissociation (CID) chamber. Then daughter ions are detected by MS detector in m/z unit. The masses are used to match in silico protein database, which is known as database search. However, this approach difficult to process a full protein characterization by current technologies because of post-translational modification (PTM).

In contrast, the top-down approach focuses on a complete analysis of proteins and their full description by fragmenting total proteins in the MS, followed by the quantification of these fragments [31, 32]. Nevertheless, this approach only feasible to study a single protein. To reduce complexity, protein from mosquitoes requires regular tissue-specific analysis and fractionate the protein complement in the main tissue sample. Reducing this complexity produces a better protein identification [19].

One and two-dimensional sodium-dodecyl sulfate (1-DE and 2-DE) gel electrophoresis (SDS-PAGE), which are gel-based protein separation techniques, have been performed to analyze the full proteome of mosquitoes. In 2-DE, proteins are separated by an isoelectric point using pH gradient (IPG) strip gel, in an electrical field. Proteins then traverse to the anode or cathode in an isoelectric focus (IEF) based on the molecular charge, until they attained an isoelectric point. Then the IPG strip is placed on top of SDS-PAGE for the 2-dimension separation based on protein molecular weight or size. The resultant proteins usually clear-up into different spots. The spots can be visualized, excised, and digested for protein quantitation and identification [32].

Mosquito proteome has uncovered modifications in the hemolymph proteins, midgut, and salivary gland proteins, peritrophic membrane proteins, and other proteins post-blood-feeding. Proteome-wide analysis has also elucidated vector-parasite interactions, susceptibility and resistant profiles to insecticides [33].

Ae. aegypti larval midgut, an organ highly specialized because it is responsible for supporting ion transport, amino acids, lipids, and sugar absorptions. It is composed of a certain layer of columnar epithelial cells resting on a basement membrane. The midgut laminar surface is enhanced by an array of microvilli, the brush border membrane, which contains digestive enzymes, ion channels, and other extracellular matrices, while a system of intracellular actin filaments maintains these appendages [34]. Research into insect membrane proteins was made possible by the preparation of the brush border membrane vesicle (BBMV) samples. The first proteome study was conducted by Popova-Butler and Dean (2009) on *Ae. aegypti* [34].

Popova-Butler and Dean (2009) analyzed *Ae. aegypti* midgut proteins focused on the most abundant proteins of the apical BBMV. The study combined two proteomics methods, two-dimensional gel electrophoresis, and a shotgun two-dimensional liquid chromatography approach. These mixed methods are primarily on multidimensional protein identification technology (MudPIT). The study revealed 36 most abundant proteins in the BBMV detected by both methods. The complementary proteomic approaches identified a total of 119 proteins; 86 by MudPIT method and 69 by 2D gel electrophoresis. Approximately, 400 spots detected on 2D gels, and 39 spots were cored and identified by MudPIT, respectively [34]. Their technique has demonstrated the efficiency of multiple protein extraction

methods and separation techniques, eventually indicating a high confidence level in the data obtained.

Cancino-Rodezno *et al.* (2012) revealed *Ae. aegypti* larval midgut intoxicated with Cry11Aa toxin from *Bti*. The study was performed by intoxicating larval midgut with two doses of Cry11Aa toxin LC_{10} and LC_{50} compared with a toxin buffered treatment. The identified proteome differences analyzed by two-dimensional differential gel electrophoresis (2D-DIGE). The highly significant differentially expressed protein spots were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS). In the LC_{10} treatment, only two protein spots identified with significantly altered expression levels, the F0F1-type ATP synthase β subunit and a serine-type endopeptidase [35].

In contrast, 22 protein spots showing significant changes in their expression levels in the LC_{50} treatment. The most abundant class of proteins identified by MS were proteins involved in protein turnover and folding, ATP production, lipid metabolism, and cell cytoskeleton maintenance. Then, RNA interference (RNAi) of the most abundant proteins was performed on HSP90 heat shock protein, actin, and ATP synthase subunit β. Once ATP synthase subunit β and actin expressions were affected by suppression with RNAi, the larvae transformed into highly susceptible to Cry11Aa toxin action. Furthermore, the suppression of HSP90 resistance was shown in the larvae [35]. In this case, the silencing of the protein action works by inhibiting protein translation and suppression of the gene post-transcriptionally.

An early midgut peritrophic matrix proteome of adult female *Ae. aegypti* was analyzed using LC-MS, and the resultant data was examined by the Vectorbase Biomart tool to reveal the predicted structural details of the proteome by Whiten *et al*. (2018). A total of 474 exclusive proteins from 6,262 peptide sequences were identified, with 115 were predicted as secreted proteins. Serine-type proteases were shown to be in abundance. Others included proteins with catalytic activity, hydrolase activity, peptidase activity, serine-type peptidase activity, and serine-type endopeptidase activity. Whiten *et al.* (2018) also isolated early trypsin, late trypsin, serine collagenase along with other 19 serine-type peptidases. Furthermore, immune-related proteins like fibrinogen and fibronectin proteins, transferrin, serpins Niemann-pick type C-2 proteins, clip-domain serine proteins, cathepsin B and L, galectins prophenoloxidase were also identified. Finally, a few novel proteins with anonymous functions were also identified [36].

4. Differential protein expressions in susceptible and resistant strains

In a recent study by Mano *et al.* (2019) in Thailand showed distinct protein expression in adult female salivary glands of pyrethroid susceptible and resistant *Ae. aegypti* strains, using 1D-PAGE and 2D- PAGE coupled with NanoLC-MS/MS, and bioinformatics tools. The study concluded that pyrethroids might likely trigger changes in the salivary gland proteins in resistant mosquitoes and it was because of the expression extent of each protein spot in the susceptible and the resistant strains, which revealed 3 downregulated proteins in resistant mosquitoes. SDS-PAGE analysis showed nine main proteins among the susceptible and the resistant strains, and one protein band at 20kDa was seen only in the susceptible strain. The 2D-PAGE analysis revealed 19 expressed proteins in both strains involved in blood-feeding processes, stress response, immunogenic response, and metabolic processes [37].

Wang *et al.* (2015) reported a quantitative proteome analysis of pyrethroid susceptible and resistant *Culex pipiens pallens* mosquitoes. For MS/MS analysis, they used Isobaric tags for relative and absolute quantitation (iTRAQ). Then followed by gene ontology analysis of the differentially expressed proteins. Finally, differentially expressed proteins in both laboratory and field strain *Cx. pipiens pallens* validated by Western blot. The study found 30 differentially expressed proteins assigned to ten different classes. These classes included: oxidoreductase activity, transporter activity, catalytic activity, structural constituent of the cuticle, transferase activity, hydrolase activity, phosphotransferase activity, DNA binding, and nucleic acid-binding functions. Furthermore, CYP6AA9 overexpressed protein was validated on the field strain mosquitoes and confirmed to be involved in pyrethroid resistance and might be a potential marker to monitor and predict the pyrethroid resistance level of a field population [38].

Djegbe *et al*. (2011) described the differential expression of salivary proteins in organophosphate susceptible and resistant mosquitoes. 2DE and MS were employed to analyze two strains of *Cx. quinquefasciatus* mosquitoes with equal genetic build-up but showing either insensitive acetylcholinesterase (ace1R resistance allele) or not (wild type). Ace1R allele is known to exert cross-resistance to organophosphate and carbamates. The study revealed four differentially expressed salivary gland proteins ($P<0.05$) in the susceptible versus the resistant strain. Primary salivary protein D7 long-form involved in blood-feeding accomplishment exhibited lower expression in the resistant strain. Meanwhile, three proteins of ace 1R resistant in the salivary glands were also significantly overexpressed [39].

5. Future Perspective

The proteomics technologies have successfully revealed several potential biological markers from insecticide resistance mosquitoes and could be used to predict resistance in the field strain. Nevertheless, researchers must test the biomarkers in different geographical areas as it could be specific to a particular area of study. Therefore, the proteomics studies on the resistance strain should be conducted in different geographic regions, and compare the results to draw the best conclusion for a suitable marker. The markers perhaps could also be utilized as a new target for the development of environmentally friendly insecticides.

6. Conclusion

Proteomics analyses using MS technologies in mosquitoes allow the full identification of the proteins in this vector. Existing proteomics methods not only decipher protein expression at different physiological conditions but also post-translational protein modification and protein-protein interactions. Validation of differentially expressed proteins in resistant mosquitoes may provide a potential biomarker for resistance monitoring, surveillance, and control strategies.

Author Disclosure statement

No competing financial interest to declare.

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8. References

1. Wilson AL, Courtenay O, Kelly-Hope LA, Scott TW, Takken W, Torr SJ *et al*. The importance of vector control for the control and elimination of vector-borne diseases. PLoS neglected tropical diseases. PloS neglected tropical diseases. 2020; 14(1):e0007831.
2. Karunamoorthi K, Sabesan S. Insecticide resistance in insect vectors of disease with special reference to mosquitoes: a potential threat to global public health, 2013.
3. Organization WH. World Malaria Report. Reference Source, 2019.
4. Achee NL, Grieco JP, Vatandoost H, Seixas G, Pinto J, Ching-NGL *et al*. Alternative strategies for mosquito-borne arbovirus control. PLoS neglected tropical diseases. 2019; 13(1):e0006822.
5. Van den Berg M, Ogutu B, Sewankambo NK, Biller-Andorno N, Tanner MRTS. S malaria vaccine pilot studies: addressing the human realities in large-scale clinical trials. Trials. 2019; 20(1):1-4.
6. Liu N. Insecticide resistance in mosquitoes: impact, mechanisms, and research directions. Annual review of entomology. 2015; 60:537-559.
7. Paine MJ, Brooke B. Insecticide resistance and its impact on vector control, in Advances in Insect Control and Resistance Management., Springer. 2016, 287-312.
8. Marcombe S, Fustec B, Cattel J, Chonephetsarath S, Thammavong P, Phommavanh N *et al*. Distribution of insecticide resistance and mechanisms involved in the arbovirus vector Aedes aegypti in Laos and implication for vector control. PLoS neglected tropical diseases. 2019; 13(12):e0007852.
9. Burns CJ, Pastoor TP. Pyrethroid epidemiology: a quality-based review. Critical reviews in toxicology. 2018; 48(4):297-311.
10. Narahashi T. Nerve membrane ion channels as the target site of insecticides. Mini reviews in medicinal chemistry. 2002; 2(4):419-432.
11. Amelia-Yap ZH, Chen CD, Sofian-Azirun M, Low VL. Pyrethroid resistance in the dengue vector Aedes aegypti in Southeast Asia: present situation and prospects for management. Parasites & vectors. 2018; 11(1):1-17.
12. Ishak IH, Jaal Z, Ranson H, Wondji CS. Contrasting patterns of insecticide resistance and knockdown resistance (KDR) in the dengue vectors Aedes aegypti and Aedes albopictus from Malaysia. Parasites & vectors. 2015; 8(1):1-13.
13. Fernando SD, Hapugoda M, Perera R, Saavedra-Rodriguez K, Black WC, De Silva NK. First report of V1016G and S989P knockdown resistant (kdr) mutations in pyrethroid-resistant Sri Lankan Aedes aegypti mosquitoes. Parasites & vectors. 2018; 11(1):526.
14. Ishak IH, Kamgang B, Ibrahim SS, Riveron JM, Irving H, Wondji CS. Pyrethroid resistance in Malaysian populations of dengue vector Aedes aegypti is mediated by CYP9 family of cytochrome P450 genes. PLoS neglected tropical diseases. 2017; 11(1):e0005302.
15. Strode C, Wondji CS, David J-P, Hawkes NJ, Lumjuan N, Nelson DR *et al*. Genomic analysis of detoxification genes in the mosquito Aedes aegypti. Insect biochemistry and molecular biology. 2008; 38(1):113-123.
16. Weedall GD, Mugenzi LM, Menze BD, Tchouakui M, Ibrahim SS, Amvongo-Adjia N *et al*. A cytochrome P450 allele confers pyrethroid resistance on a major African malaria vector, reducing insecticide-treated bednet efficacy. Science translational medicine. 2019; 11(484).
17. Balabanidou V, Grigoraki L, Vontas J. Insect cuticle: a critical determinant of insecticide resistance. Current opinion in insect science. 2018; 27:68-74.
18. Huang Y, Guo Q, Sun X, Zhang C, Xu N, Xu Y *et al*., Culex pipiens pallens cuticular protein CPLCG5 participates in pyrethroid resistance by forming a rigid matrix. Parasites & vectors. 2018; 11(1):1-10.
19. Hugo RLE, Birrell GW. Proteomics of anopheles vectors of malaria. Trends in parasitology. 2018; 34(11):961-981.
20. Gillet LC, Leitner A, Aebersold R. Mass spectrometry applied to bottom-up proteomics: entering the high-throughput era for hypothesis testing. Annual review of analytical chemistry. 2016; 9:449-472.
21. Sehrawat N, Gakhar SK. Mosquito proteomics: present and future prospective. Research in Biotechnology. 2014; 5(4).
22. Popova-Butler A, Dean DH. Proteomic analysis of the mosquito Aedes aegypti midgut brush border membrane vesicles. Journal of insect physiology. 2009; 55(3):264-272.
23. Cancino-Rodezno A, Lozano L, Oppert C, Castro JI, Lanz-Mendoza H, Encarnación S *et al*. Comparative proteomic analysis of Aedes aegypti larval midgut after intoxication with Cry11Aa toxin from Bacillus thuringiensis. PloS one. 2012; 7(5):e37034.
24. Whiten SR, Ray WK, Helm RF, Adelman ZN. Characterization of the adult Aedes aegypti early midgut peritrophic matrix proteome using LC-MS. PloS one. 2018; 13(3):e0194734.
25. Mano C, Jariyapan N, Sor-Suwan S, Roytrakul S, Kittisenachai S, Tippawangkosol P *et al*., Protein expression in female salivary glands of pyrethroid-susceptible and resistant strains of Aedes aegypti mosquitoes. Parasites & vectors. 2019; 12(1):111.
26. Wang W, Lv Y, Fang F, Hong S, Guo Q, Hu S *et al*. Identification of proteins associated with pyrethroid resistance by iTRAQ-based quantitative proteomic analysis in Culex pipiens pallens. Parasites & vectors. 2015; 8(1):1-11.
27. Djegbe I, Cornelie S, Rossignol M, Demettre E, Seveno M, Remoue F *et al*. Differential expression of salivary proteins between susceptible and insecticide-resistant mosquitoes of Culex quinquefasciatus. PLoS One. 2011; 6(3):e17496.
28. Ranson H, Jensen B, Vulule J, Wang X, Hemingway J, Collins F. Identification of a point mutation in the voltage-gated sodium channel gene of Kenyan Anopheles gambiae associated with resistance to DDT and pyrethroids. Insect molecular biology. 2000; 9(5):491-497.
29. Brengues C, Hawkes NJ, Chandre F, McCarroll L, Duchon S, Guillet P *et al*. Pyrethroid and DDT cross-resistance in Aedes aegypti is correlated with novel mutations in the voltage-gated sodium channel gene. Medical and veterinary entomology. 2003; 17(1):87-94.
30. Enayati AA, Vatandoost H, Ladonni H, Townson H, Hemingway J. Molecular evidence for a kdr-like

pyrethroid resistance mechanism in the malaria vector mosquito Anopheles stephensi. Medical and veterinary entomology. 2003; 17(2):138-144.

31. Xu Q, Liu H, Zhang L, Liu N. Resistance in the mosquito, Culex quinquefasciatus, and possible mechanisms for resistance. Pest Management Science: formerly Pesticide Science. 2005; 61(11):1096-1102.
32. Saavedra-Rodriguez K, Urdaneta-Marquez L, Rajatileka S, Moulton M, Flores A, Fernandez-Salas I *et al*. A mutation in the voltage-gated sodium channel gene associated with pyrethroid resistance in Latin American Aedes aegypti. Insect molecular biology. 2007; 16(6):785-798.
33. Chang C, Shen W-K, Wang T-T, Lin Y-H, Hsu E-L, Dai S-M. A novel amino acid substitution in a voltage-gated sodium channel is associated with knockdown resistance to permethrin in Aedes aegypti. Insect biochemistry and molecular biology. 2009; 39(4):272-278.
34. Yanola J, Somboon P, Walton C, Nachaiwieng W, Prapanthadara L-A. A novel F1552/C1552 point mutation in the Aedes aegypti voltage-gated sodium channel gene associated with permethrin resistance. Pesticide Biochemistry and Physiology. 2010; 96(3):127-131.
35. Liu N, Li T, Reid WR, Yang T, Zhang L. Multiple cytochrome P450 genes: their constitutive overexpression and permethrin induction in insecticide resistant mosquitoes, Culex quinquefasciatus. Plos one. 2011; 6(8):e23403.
36. Kushwah RBS, Dykes CL, Kapoor N, Adak T, Singh OP. Pyrethroid-resistance and presence of two knockdown resistance (kdr) mutations, F1534C and a novel mutation T1520I, in Indian Aedes aegypti. PLoS Negl Trop Dis. 2015; 9(1):e3332.
37. Ishak IH, Riveron JM, Ibrahim SS, Stott R, Longbottom J, Irving H *et al*. The Cytochrome P450 gene CYP6P12 confers pyrethroid resistance in kdr-free Malaysian populations of the dengue vector Aedes albopictus. Scientific reports. 2016; 6:24707.
38. Zhao L, Alto BW, Shin D, Yu F. The effect of permethrin resistance on Aedes aegypti transcriptome following ingestion of Zika virus infected blood. Viruses. 2018; 10(9):470.
39. Fadel AN, Ibrahim SS, Tchouakui M, Terence E, Wondji MJ, Tchoupo M *et al*. A combination of metabolic resistance and high frequency of the 1014F kdr mutation is driving pyrethroid resistance in Anopheles coluzzii population from Guinea savanna of Cameroon. Parasites & vectors. 2019; 12(1):1-13.