First Report of F1534C kdr Mutation in Deltamethrin Resistant *Aedes Albopictus* From Northern Part of West Bengal, India

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Article

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Abstract

West Bengal is a dengue endemic state of India with several dengue outbreaks in the recent years. Synthetic pyrethroids are the major arsenal used for the control of these vectors. The emergence of pyrethroid resistance in mosquitoes has recently been described from many parts of the world due to kdr (knock down resistance) mutations in the voltage gated sodium channel gene (*vgsc*). In *Aedes albopictus*, at least four such mutations had already been found. In this study, wild populations of *Ae. albopictus* were sampled from different locations of northern part of West Bengal, India. World Health Organization, 2016 recommended adult bioassay was followed throughout the experiment in order to determine susceptibility status. A total of 200 *Ae. albopictus* specimens including both phenotypically resistant and susceptible individuals were successfully amplified for F1534C kdr genotyping. Among them, 81% were homozygote susceptible,12.5% were heterozygote and 6.5% were homozygote resistant. Presence of F1534C mutation in pyrethroid resistant *Ae. albopictus* mosquitoes is the first report from India. Moreover, another novel mutation T1520I was also found to be coexisting with F1534C mutation in *Ae. albopictus*. This study will aid in identifying insecticide resistance mechanism and therefore, will reduce errors in vector control measures.

1.introduction

The Asian tiger mosquito, *Aedes albopictus* (Skuse) (Diptera: Culicidae), is an epidemiologically important vector for the transmission of a variety of viral infections such as dengue fever, chikungunya, yellow fever, and zika. Dengue is the most rapidly spreading vector-borne disease and has thus emerged as a potential threat to global public health and the global economy, particularly in tropical and subtropical countries such as India[1].Additionally, *Ae. albopictus* has been linked to zika virus infections in the large extent of India from recent past[2].West Bengal is a dengue endemic state of India and all four dengue virus serotypes has been identified from this region[3]. Northern part of West Bengal has a congenital environment (temperature and relative humidity) for the growth and proliferation of *Aedes* mosquitoes,resulting several dengue outbreaks in the recent years[4].Due to emergence and spread of new dengue serotypes and lack of protective vaccines, vector control is the sole way for global management of mosquito borne diseases and use of chemical insecticide is the prime armament in vector population reduction[5]. As the uses of chemical insecticides increased sharply, development of resistance against these insecticides occurs simultaneously. Mosquitoes have acquired insecticide resistance as a result of the indiscriminate application of insecticides directly targeted at them, as well as indirect exposure to insecticides sprayed on agricultural fields[6]. One of the major ways of gaining such resistance is the target site insensitivity in which the targeted site for insecticide is altered through point mutations. Several mutations had been identified from mosquito species that served as a primary machinery to develop strong resistance against dieldrin, carbamates, DDT (Dichlorodiphenyltrichloroethane) and synthetic pyrethroids[7–10]. In India, DDT, malathion, deltamethrin, lambda-cyhalothrin, cyfluthrin, alpha-cypermethrin, bifenthrin, and cyphenothrin are used under the National Center for Vector Borne Diseases Control (NCVBDC) for control of malaria and other vector

borne diseases [11]. Among them largely used DDT and synthetic pyrethroids target voltage gated sodium channel in mosquito vectors, altering its gating properties and finally producing a *knockdown* effect[10].The channel is a transmembrane protein in neuronal axons, contains four homologous repeats(I-IV), each with six transmembrane segments (S1-S6) with a circular radial arrangement in which a central ion pore is formed[12]. Earlier research on *Ae. albopictus* from various countries found a wide range of insecticide resistance as well as the existence of kdr mutations, particularly at IIS6 segment 1016 and IIIS6 segment 1532 and 1534 of the *vgsc* gene[13, 14]. DDT and synthetic pyrethroid resistance have also been observed in *Ae. albopictus* populations from several parts of India, although no kdr mutation has been discovered till date [15].Given the global rise of pyrethroid resistance and emergence of kdr mutation in the vector populations it was critical to investigate the status of resistance and the presence of probable kdr mutations in Indian *Ae. albopictus* populations. The current research focuses on the mapping of major kdr mutation in wild population of *Ae. albopictus* and their role in DDT and synthetic pyrethroid resistance in the northern parts of west Bengal, India.

2. Results

2.1. Demography of the study area

Darjeeling is the northernmost district of West Bengal located in foothills of Himalayas and sharing international boundaries with Nepal and Bangladesh. This research was carried throughout Darjeeling district's several blocks, including rural, urban, and semi-urban areas. The average larval density at different sampling sites indicates that there are plenty of mosquito breeding habitats. Details of the mosquito collection, climatic conditions of sampling sites, co-existence of other species and nature of habitats are summarized in Supplementary Table S1.

2.2. Insecticide resistance profile of Ae. albopictus

The study of adult bioassay revealed that multiple resistance was developed in the wild population of *Ae. albopictus* against DDT and synthetic pyrethroids. Mortality percentage of the studied mosquito populations against DDT, permethrin, lambda-cyhalothrin and deltamethrin was shown in Table 1. Mortality percent ranged from 75.77–83.30 for DDT, 86.43–93.43 for permethrin, 78.7-88.28 for lambda-cyhalothrin and 77.09–83.97 for deltamethrin. Highest level of resistance was observed against DDT in NAX^{al}, BAG^{al}, and MAT^{al} populations whereas SUK^{al} shows highest resistance against lambda-cyhalothrin. Mosquitoes from all the study site are confirmed resistant against all the tested insecticide except the two populations *i.e.*, NAX^{al} and SUK^{al} were found to be probable resistant against permethrin. Among synthetic pyrethroids, lowest mortality was found in deltamethrin followed by lambda-cyhalothrin and permethrin except in SUK^{al} population where lambda-cyhalothrin showed least mortality.

Table 1
Insecticide resistance profile of *Ae. albopictus* (n ≥ 100) from northern part of West Bengal, India against DDT and synthetic pyrethroids. M%-Mortality percentage; S.E-Standard Error; n-total number of adult mosquitos.

Mosquito Population	DDT M % ± S.E	PERMETHRIN M % ± S.E	LAMBDA-CYHALOTHRIN M % ± S.E	DELTAMETHRIN M % ± S.E
NAX^{al}	76.45 ± 0.43	93.15 ± 0.87	88.28 ± 0.40	77.56 ± 0.57
SUK^{al}	83.30 ± 0.35	93.43 ± 1.15	78.7 ± 0.42	83.97 ± 0.41
BAG^{al}	77.36 ± 0.40	88.99 ± 1.01	81.23 ± 0.41	77.09 ± 0.70
MAT^{al}	75.77 ± 0.87	86.43 ± 0.49	83.76 ± 0.82	79.97 ± 1.17

2.3. Knockdown Rates

The knock down times (KDT_{10}, KDT_{50}, KDT_{95}) against the tested insecticides was shown in Supplementary Table S2. NAX^{al} population showed highest KDT values against DDT. Against permethrin highest KDT_{95}value was recorded from MAT^{al} population whereas highest KDT_{10} and KDT_{50} found in NAX^{al} population. MAT^{al} population also showed highest KDT_{95} and KDT_{50} values against lambda-cyhalothrin. NAX^{al} and SUK^{al} populations had a higher KDT values against deltamethrin. Such high KDT values indicated that the various insecticides took a long time to knock down 10%, 50%, and 95% of the *Ae. albopictus* population, depicting the emergence of resistance.

2.4. F1534C kdr genotyping of Ae. albopictus

A total of 200 specimens of *Ae. albopictus* from four sampling sites was successfully amplified and all three genotypes were identified (Fig. 1). Among them, 162 (81%) were susceptible (1534 F/F), 25 (12.5%) were heterozygote resistant (1534 F/C), and 13 (6.5%) were homozygote resistant (1534 C/C). Only one population *i.e.*, MAT^{al} was found to carry mutated Cystine allele (Fig. 2). The frequency of C allele in deltamethrin resistance and deltamethrin susceptible populations were found to be 0.58 and 0.44 respectively (Supplementary Table S3). Kdr genotyping demonstrate that three out of the four population *i,e.*, BAG^{al}, NAX^{al} and SUK^{al} were exclusively homozygous for F allele. In the MAT^{al} population, all three genotypes (1534F/F, 1534F/C and 1534C/C) were found for the kdr locus from both phenotypically resistant and susceptible mosquitoes. Of the three different genotypes, 50% were heterozygous (1534F/C), 26% were homozygous resistant (1534C/C) and 24% were homozygous susceptible (1534F/F). The genotype frequencies at kdr locus for deltamethrin resistance and deltamethrin susceptible population followed the Hardy Weinberg Equilibrium (HWE) ($P < 0.05$) (Table 2). The de Finetti diagram (Fig. 3) of genotype frequencies of MAT^{al} population reveals the exact distribution pattern of kdr genotypes and the deviation from HWE.

Table 2
Distribution of knockdown resistance genotypes in relation to Hardy-Weinberg proportion for deltamethrin resistance and deltamethrin susceptible *Ae. albopictus* from northern part of West Bengal, India.

MAT^{al} mosquito population			**Deltamethrin Resistance**	**Deltamethrin Susceptible**
Genotypes	FF	Observed No.	**5**	**7**
		Expected No.	**4.41**	**7.84**
		Chi-square (χ^2)	**0.078**	**0.09**
	FC	Observed No.	**11**	**14**
		Expected No.	**12.18**	**12.32**
		Chi-square (χ^2)	**0.114**	**0.229**
	CC	Observed No.	**9**	**4**
		Expected No.	**8.81**	**4.84**
		Chi-square (χ^2)	**0.004**	**0.145**
Allele Frequency	F		**0.42**	**0.56**
	C		**0.58**	**0.44**
Deviation from Hardy Weinberg Equilibrium	Inbreeding Coefficient (F)		**0.09688**	**-0.13636**
	Pearson's Chi-square(df = 1)		**χ^2 = 0.196 p-value = 0.628102**	**χ^2= 0.464 p-value = 0.495354**
	Exact Test		**p-value = 0.686793**	**p-value = 0.691113**

2.5. Dna Sequence Analysis

An amplified 350 bp fragments (Supplementary Fig. 1) of IIIS6 *vgsc* gene were sequenced and the sequences were submitted to GenBank database (Accession No. OM421596 and OM421597). A total of six samples from MAT^{al} population were sequenced to check efficiency of AS-PCR assay and to confirm the presence of F1534C kdr mutation in *Ae. albopictus*. Sequence alignment (Fig. 4) with other homologous sequences obtained from the study in Brazil and Japan showed the presence of both Phenylalanine (TTC) and Cystine (TGC) at 1534 codon of IIIS6 *vgsc* site of *Ae. albopictus* population. Another mutation in the 1520 codon of IIIS6 *vgsc* gene from Threonine (ACC) to Isoleucine (ATC) was also found in association with F1534C kdr in wild population of *Ae. albopictus.*

3. Discussion

The present study revealed that an enhanced level of insecticide resistance was prevalent among the wild populations of *Ae. albopictus* along with kdr mutation from the dengue endemic district of northern part of West Bengal, India. Average larval density from different sampling site gives strong support for the occurrence of dengue from this region [16]. *Armigeres sp*. was found in large number to share habitat with *Aedes* larvae specially in the higher altitude areas, which may causes Zika viral disease as it is reported from China [17]. Earlier study in *Ae. albopictus* from the northern part of West Bengal exhibited confirm resistance against DDT but were susceptible to synthetic pyrethroids (deltamethrin and lambda-cyhalothrin) and organophosphate (malathion)[18, 19]. Occurrence of several dengue outbreaks in the recent time gives major push to the authority for applying more insecticides (alpha-cypermethrin for adult and temephos for larvae) specially in the urban and semi urban areas of the district (personal communication). In comparison to *Ae. aegypti*, resistance levels in *Ae. albopictus* were low, presumably because this exophilic species experience lower exposure to insecticides, particularly those targeting the adult stage[20]. Current study included both types of (type I and type II) synthetic pyrethroids. All the studied mosquito populations are resistant against DDT, lambda-cyhalothrin and deltamethrin (Table 1). Two out of four populations (NAX^{al} and SUK^{al}) exhibited probable resistance against permethrin. As the type II pyrethroids were most frequently used for mosquito control programme in West Bengal, that might reflect in the development of more resistance against type II pyrethroids as compared to type I. Increased knockdown times against tested insecticides (Supplementary Table S2) also point to the emergence of resistance. An elevated level of different detoxifying enzymes *i,e*, Cytochromes P450, Carboxylesterases and Glutathione S-transferase were earlier detected in *Ae. albopictus* population from this northern part of West Bengal and it was believed to be the primary machinery behind the resistance development [18]. Study from this region found 11 synonymous and one non-synonymous mutations in the *vgsc* gene, however none of the major kdr was found [19]. Thus, in the present study AS-PCR assay was done for the screening of F1534C kdr mutation in wild population of *Ae. albopictus* and was observed that mutant Cystine allele frequency ranged from 0.44 to 0.58. The majority of F1534C mutations have been found to be heterozygous. It is clear that the introduction of the kdr mutation in these mosquito populations has only recently begun, as has been observed in other parts of the world[21]. Partial sequencing of IIIS6 *vgsc* gene further confirm one polymorphic site (TTC to TGC) at 1534 codon on exon no 31, which causes a change in phenylalanine to cystine as previously reported in *Ae. albopictus* populations from Singapore, China and Greece [22–24]. Studies in United States and China suggested that, the position of 1534 at *vgsc* gene is very changeable due to the existence of various mutations like 1534L and 1534S[23–25].Studies in *Aedes aegypti* from India also suggested that, F1534C mutation confers a high level of protection against DDT but only a moderate level of protection against deltamethrin[26].The same mutation F1534C was also reported to be associated with Type I pyrethroid permethrin resistance in *Ae. aegypti* population from Grand Cayman, where another mutation V1016I co-existed[27].Another mutation I1532T was discovered in an *Ae. albopictus* population from Italy and China, two locations upstream from the 1534 site, strengthening the importance of site-specific conditions (such as climate, disposable breeding places, techniques, and pest management frequency) in the evolution of resistant mosquito

populations[24]. Sequence analysis from present study found another novel single nucleotide polymorphism at 1520 codon of *vgsc* gene (ACC to ATC) resulting T1520I kdr mutation in wild population of *Ae. albopictus* from India. However, scarcity of data on 1520 kdr site of IIIS6 *vgsc* gene, further analysis was not allowed. Though the role of T1520I kdr mutation is not clear but this mutation has always been identified in association with the F1534C mutation[26]. Thus, the T1520I kdr mutation was hypothesized to be a compensating mutation to minimize the fitness cost of the F1534C mutation's possibly harmful effect, despite laboratory findings that *Ae. aegypti* homozygous for the F1534C mutation has no reduced fitness[28]. In the current study, both resistant and susceptible allelic genotypes were obtained from phenotypically susceptible mosquitoes. Moreover, heterozygous kdr alleles also be found in both susceptible and resistant mosquitoes from the MAT^{al} population (Supplementary Table S3). The same type of observations was also found in the *Culex quinquefasiatus* population from the North-Eastern part of India [29]. Thus, it become difficult to conclude a link between presence of kdr mutation and insecticide resistance status. Given that kdr is a recessive trait, in deltamethrin resistance MAT^{al} population the occurrence of both heterozygous (1534F/C) and homozygous susceptible (1534F/F) *Aedes* individuals suggest that other mechanisms are also involved in resistance development[30]. Thus, findings from the present study suggested that wild population of *Ae. albopictus* from northern part of West Bengal possesses both kdr mutation and increased expression of detoxifying enzymes for resistance development against routinely used insecticides. This is due to erroneous applications of large amounts of insecticide and repeated applications of the same insecticide over a lengthy period of time. This has a strong consequence on the authority to be more cautious when using insecticides and to implement more alternative vector control tactics, such as the release of Wolbachia-infected male mosquitoes or more uses of biological insecticide such as *Bti* which are becoming increasingly crucial in the current world situation [31, 32].

4. Materials And Method

4.1. Ethics statement

As the present study did not involve any human trial or higher vertebrates, the Institutional Animal Ethics Committee (IAEC) Department of Zoology, University of North Bengal (Regn. no. 840/GO/Re/S/04/CPCSEA) granted a waiver for ethics approval. The use of rat for blood feeding was also approved by the IAEC (approval no. IAEC/NBU/2019/19). All procedures were performed in accordance with relevant guidelines of the IAEC and ARRIVE (Animal Research: Reporting of In Vivo Experiments).

4.2. Study area and Sample collection

Dengue endemic Darjeeling district from northern part of West Bengal was surveyed and four different sites were selected for sampling. Larvae and pupae were collected from different breeding places and were transferred to plastic containers and brought to the laboratory. In the laboratory, mosquito larvae and pupae were reared up to F1 generation under controlled conditions (temperature 27°C±2°C; relative

humidity 75%±10%). Standard identification key of larva and adult mosquitoes was used to identify the field population up to species level [33]. The sampling was done during June to November in 2020 and March to September in 2021. Since all the sampling was done from private land, prior permission was taken from the land owner for mosquito collection.

4.3. Insecticides used

Insecticide impregnated papers (4% DDT, 0.05% deltamethrin, 0.05% lambda-cyhalothrin, 0.75% permethrin) used for adult bioassay were purchased from Vector control unit, Universiti Sains Malaysia.

4.4. Insecticide susceptibility bioassay

The WHO (World Health Organization) adult bioassay protocol was followed for the detection of susceptibility status of the mosquito populations [34]. Seven replicates of 20 active 3-5 days non-blood fed female mosquitoes from each population were exposed to insecticide impregnated paper for an hour and were transferred to a retention tube containing cotton balls soaked in 10% sucrose solution and maintained at laboratory condition. For control, mosquitoes were placed in tubes containing paper impregnated with silicone oil. Mortality percentage was recorded after 24 hours post-exposure and was repeated thrice for every insecticide. In order to calculate the knockdown time (KDT), knocked down mosquitoes were counted after every 10 min during one hour insecticide exposure as per previous protocol [6]. The live and dead mosquitoes obtained from the adult bioassays were kept at -20°C and employed for DNA isolation.

4.5. Extraction of genomic DNA

Genomic DNA was extracted from both the resistant and susceptible mosquitoes following the High Salt protocol with minor modifications as described previously [35]. Individual mosquito was homogenized using digestion buffer and further incubated with proteinase K at 55-60 *°C* for at least two hours. Afterwards, a 25:24:1 mixture of phenol, chloroform, and isoamyl alcohol was added to facilitate the partitioning of lipids and cellular debris into the organic phase, while isolated DNA remained in the aqueous phase. Following centrifugation, the aqueous phase containing the purified DNA was transferred to a clean tube for further analysis. Purity of the extracted DNA was checked by the SPECTROstar Nano fast scanning UV-Visible Microplate Reader (Make-BMG Labtech, Germany). DNA with an OD_{260}/OD_{280} value between 1.8 - 2 was selected for kdr genotyping.

4.6. Allele-specific PCR (AS-PCR) assay for F1534C kdr mutation

DNA stock solutions were prepared at a concentration of 25 ng/µl and used for AS-PCR genotyping. The Polymerase chain reaction (PCR) involved one reverse primer: 5'-TCT GCT CGT TGA AGT TGT CGA T-3' used for both alleles, and two forward allele-specific primers: 1534Phe: 5'-GCG GGC TCT ACT TTG TGT TCT TCA TCA TAT T-3' and 1534Cys ^{kdr} allele: 5'-GCG GGC AGG GCG GCG GGG GCG GGG CCT CTA CTT TGT GTT CTT CAT CAT GTG-3' with an annealing temperature of 60°C [13]. Each reaction was performed

in a 25 µl volume consisting of 1.5 mM $MgCl_2$, 1x PCR buffer (Promega, USA), 0.25 µM common reverse primer, 0.125 µM each mutation specific primer, 200 µM dNTP mixture (Promega, USA), 0.2 units Taq polymerase (Promega, USA) and 25 ng genomic DNA. The thermal cycling condition was set with an initial DNA denaturation step for two minutes at 94°C, followed by 35 cycles of denaturation for 30 sec at 94°C, annealing for 30 sec at mentioned temperature and extension at 30 sec at 72°C. PCR amplification products were loaded onto a 4% agarose gel and run for 1 hour at 100 V in TAE buffer and visualized by ethidium bromide staining under UV light. Since the primer used had GC tails of varying lengths, amplified products could be differentiated by base pair size.

4.7. Amplification and sequencing of IIIS6 *vgsc* gene of *Ae. albopictus*

PCR reaction was carried out for the partial amplification of IIIS6 *vgsc* gene. Primers used for these reactions were AaEx31P (5'-TCG CGG GAG GTA AGT TAT TG-3',) and AaEx31Q (5'-GTT GAT GTG CGA TGG AAA TG-3',) [13]. Reaction was carried out with 1X Go®Taq G2 Green Master Mix (Promega, USA) of 12.5µl, 1 µl of both forward and reverse primers, 2 µl of template DNA and 8.5 µl of nuclease free water in 25 µl reaction mixture. PCR condition was: 95 °C for 5 min, followed by 35 cycles of 95 °C for 30 s, 60 °C for 40 s and 72 °C for 1 min with a final extension step at 72 °C for 5 min. The amplified fragments of the expected size were purified using ExoSAP following manufacturer recommendations and were sequenced (Heredity Lifesciences Pvt. Ltd. Patia, Bhubaneswar-751024, Odisha, India). The sequences were analyzed with BioEdit software (v 7.0.9) and aligned with different homologous regions of *vgsc* gene sequences (KX371864, KX371865, and AB827824) of *Ae. albopictus* available in Gene Bank by using ClustalW software (v 2.0) [36,37].

4.8. Data Analysis

Mean mortality percentage against all the tested insecticides were calculated by using kyPlot 6.0. In WHO adult bioassays, control mortalities were below 10%, so no calculation of corrected mortality was needed. WHO 2016 criteria were followed to determine the resistance /susceptibility status [S=Susceptible (Mortality percentage=98-100%); R=Confirm Resistance (Mortality percentage < 90%); PR=Possible Resistance (Mortality percentage=90-98%)]. Knockdown times were determined by performing probit regression analysis in IBM SPSS (v21.0) at 95% confidence level. The web-based programme 'de FINETTI generator' version (v3.0.5) (2008) (https://finetti.meb.uni-bonn.de/) was used to compute genotype frequencies and their deviation from the Hardy-Weinberg equilibrium (HWE), which was shown within the de Finetti diagram. The diagram includes a triangular plot which represent the distribution of three genotypes in reference to one another. The curved line in the diagram represents the Hardy-Weinberg parabola that indicates the sites where alleles are in a state of HWE. The chi-square test is used to calculate the significance of the distance between the parabolic curve and the genotypes, which reflects the extent of divergence from the HWE.

5. Conclusions

The current insecticide susceptibility status of the wild population of *Ae. albopictus* from northern part of West Bengal, India was reported in this study. Furthermore, this is the first report of the F1534C kdr mutation in the wild population of *Ae. albopictus* together with T1520I kdr mutation from India that we are aware of. The occurrence of the kdr mutation in the natural population of *Ae. albopictus* in India is a clear indication that the resistance monitoring programme should be reviewed and an alternative vector control method should be used. Findings of this study could be used as a starting point for additional research and the development of effective insecticide-based interventions against *Ae. albopictus* population of India.

Declarations

Data availability

The nucleotide sequences generated and/or analyzed during the current study are available in GenBank (accession number OM421596 and OM421597").

Competing interest

The authors declare no conflict of interest.

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Authors' contributions

M.P.M collected samples, analyzed and interpreted data, wrote the manuscript and performed all the experiments. D.S designed the study and supervised the work. All authors read and approved the final manuscript.

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Figures

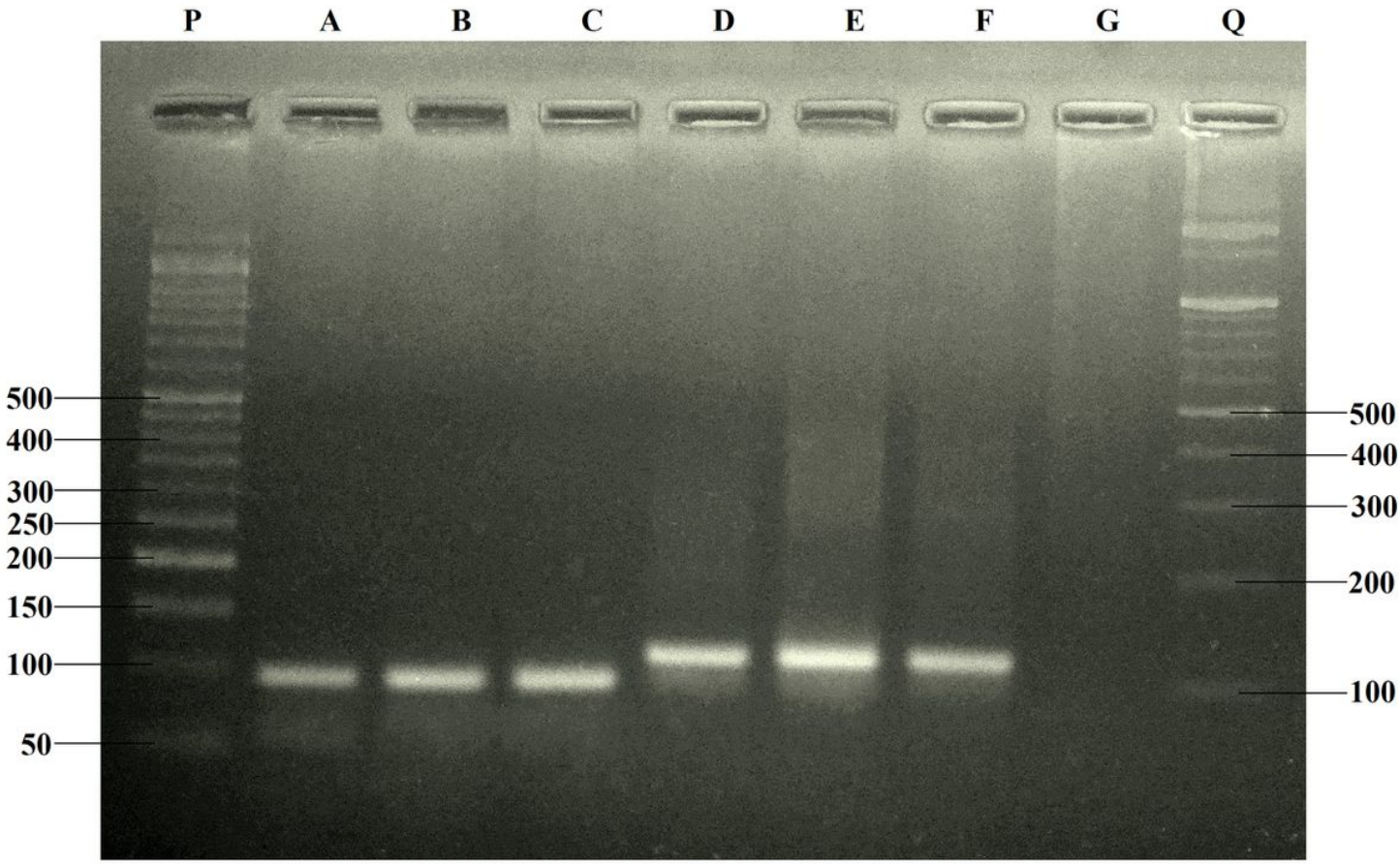

Figure 1

Gel electrophoresis image showing characteristic 93 and 113 bp bands obtained through allelic-specific PCR (AS-PCR) of F1534C kdr mutation in *vgsc* gene in *Ae. albopictus* from northern part of West Bengal, India. Lane P: 50-1500 bp DNA ladder, Lane Q: 100-1500 bp DNA ladder, Lane A, B: FF genotype, Lane C, D: FC genotypes, Lane E, F: CC genotype and Lane G: negative control.

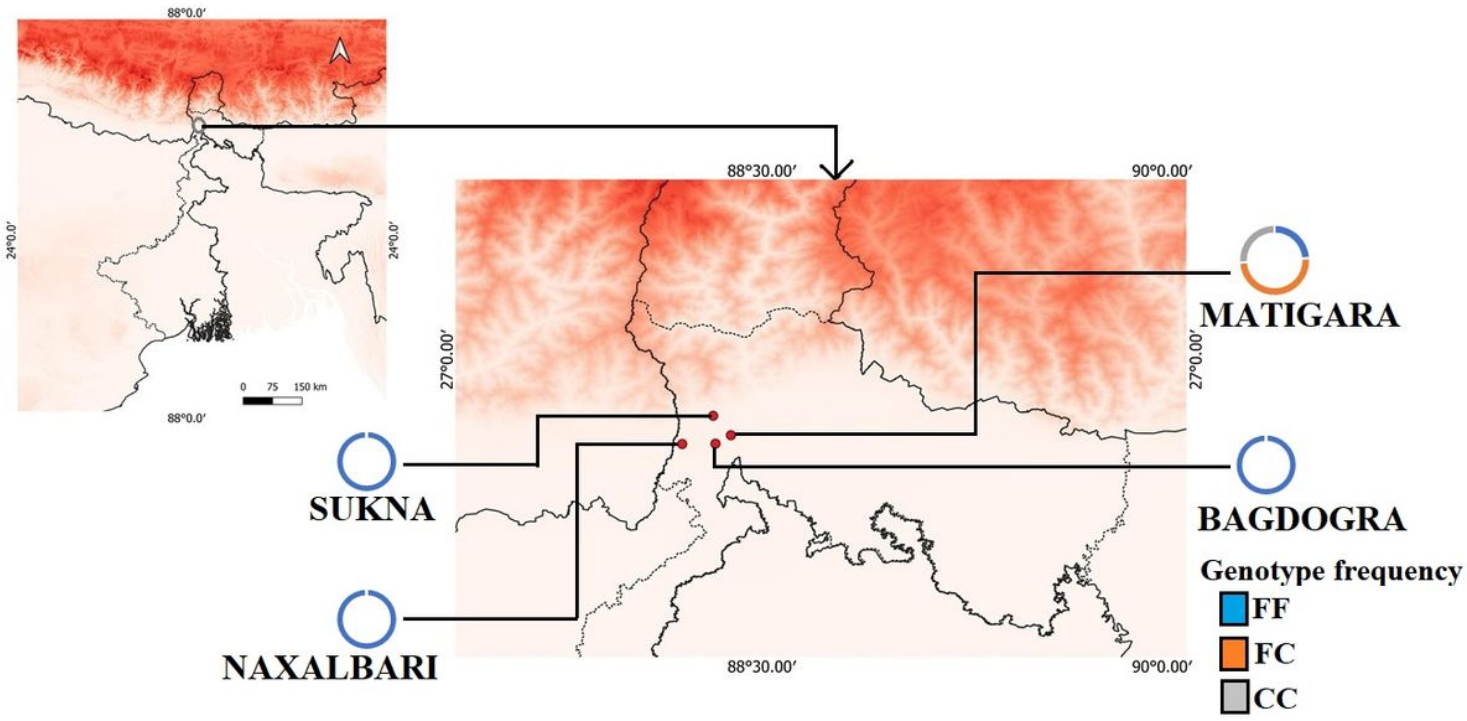

Figure 2

An altitude gradient map depicting the sampling site for *Ae. albopictus* from the northern part of West Bengal, India. The pie charts represent the percentage of kdr genotypes in *Ae. albopictus* population from the sampling site. The data were plotted on a shape file map (DIVA-GIS, https://www.diva-gis.org/gdata) using QGIS 3.16 (https://www.qgis.org/ja/site/forusers/ download.html).

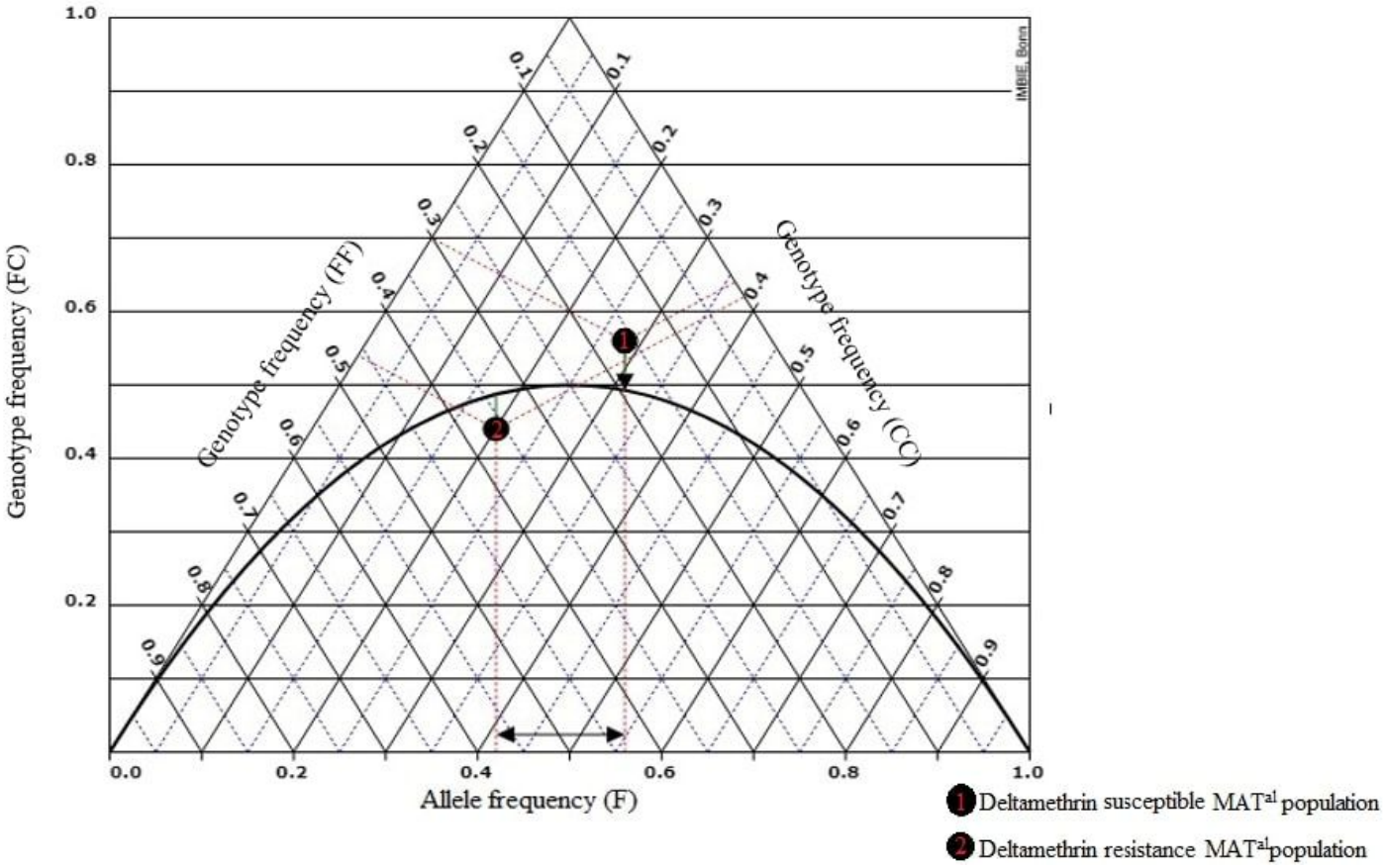

Figure 3

Hardy Weinberg equilibrium (HWE) parabola for deltamethrin resistant (1) and deltamethrin susceptible (2) populations of *Ae. albopictus* investigated for kdr genotypes, as shown in a De Finetti diagram. The length of the vertical line represented the frequency of genotype FC, the length of the left perpendicular line represented the frequency of genotype FF, the length of the right perpendicular line represented the frequency of genotype CC, the x-axis represented the frequency of allele 'F', and the Hardy–Weinberg parabola represented the point where the alleles are in Hardy–Weinberg equilibrium.

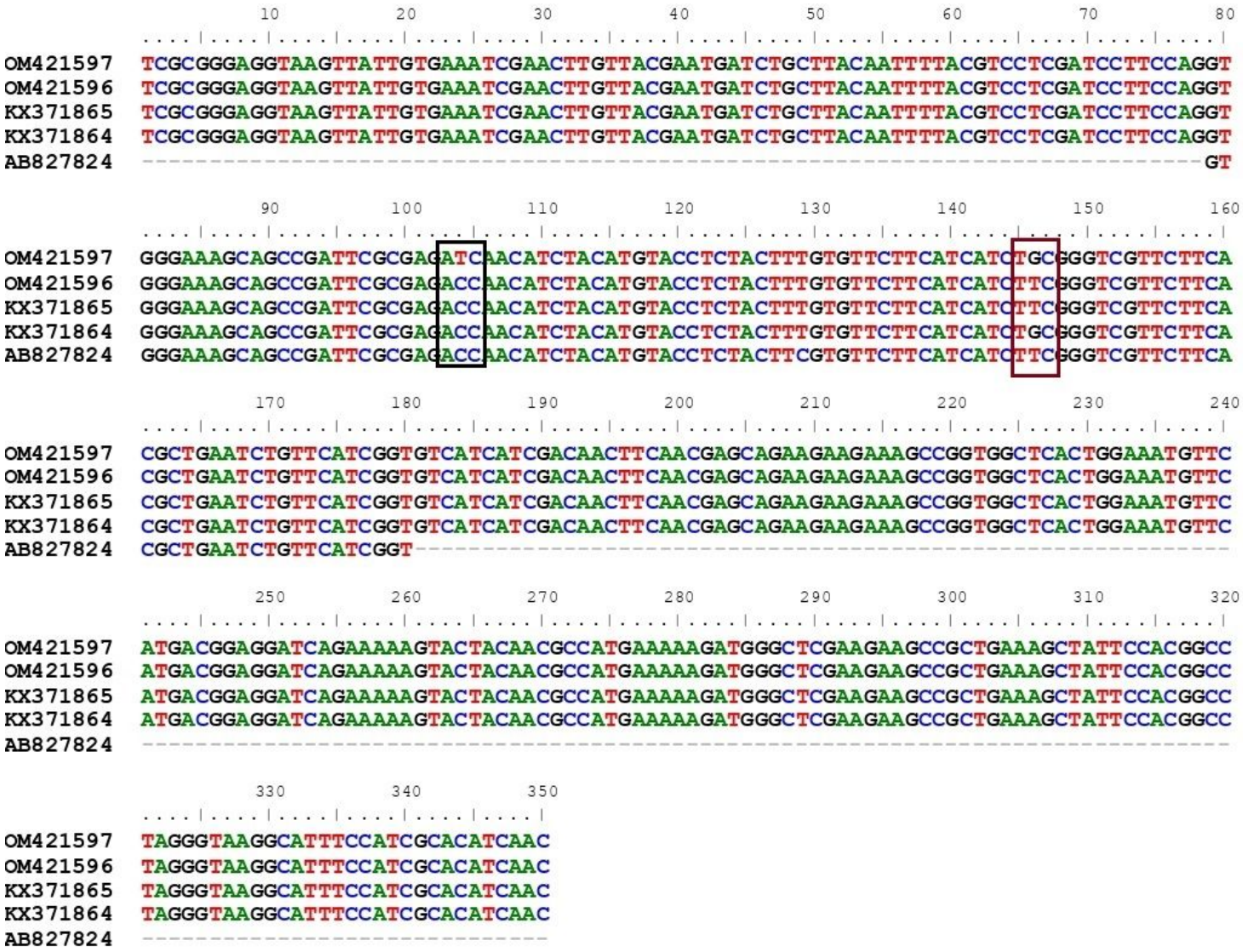

Figure 4

Nucleotide diversity in the IIIS6 *vgsc* gene sequence of *Ae. albopictus* from northern part of West Bengal. Codon 1520 and codon 1534 was indicated through black and red colour rectangular respectively.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Supplementaryfile.pdf