

Insecticide resistance and underlying targets-site and metabolic mechanisms in *Aedes aegypti* and *Aedes albopictus* from Lahore, Pakistan

Rafi Ur Rahman, Barbara Souza, Iftikhar Uddin, Luana Carrara, Luiz Paulo Brito, Monique Melo Costa, Muhammad Asif Mahmood, Sozaina Khan, Jose Bento Pereira Lima, Ademir Jesus Martins

A - *Aedes aegypti*

Aedes aegypti - *Nav* gene - IIS6 segment, exons 20-21

Pakistani population - IIS6 haplotypes

```

                                     989
      N V D R F P D K D L P R W N F T D F M H S F M I V F R V L C G E W I E S M W D C [993]
#PAg_2s6_1 ACAATGTGGATCGCTTCCCGGACAAGGACCTGCCACGGTGGAACTTCACCGACTTCATGCACTCATTCATGATCGTGTTCGGGTATTGTGCGGCGAGTGGATCGAATCCATGTGGGATT [120]
#PAg_2s6_2 .....

      M L V G D V S C I P F F L A T V V I G N L V [1015]
#PAg_2s6_1 GTATGCTTGTGGGTGACGTGTCCCTGATTCCGTTCTTTTGGCCACCGTAGTGATAGGAAATCTAGTAgtaagtattccgttttgaagttcatctgttaaggtgactgaaagtaaattgg [240]
#PAg_2s6_2 .....g.....a.....g.....

#PAg_2s6_1 agcgcaacacagacttattatgtctgttaattcgtgtattcaactagtttacaaaagaccgttgatcttgatagacatcaatattagaggcgtgtagacagcgagcgaggggcgtaccaatttac [360]
#PAg_2s6_2 ..t.....g.....t.....C.C.....

                                     1016
#PAg_2s6_1 ttttagtcagtcttttcttgcattctttctttcgtgtctaaccgacaaattgttttccactcgcacagaGTACTTAACTTTTCTTAGCCTTGCTTTTGTCCAATTCGGTTCATCCTCGCTGTCGG [480]
#PAg_2s6_2 ....g.....a.....C.....

      P T A D N E T N K I A E A F N R I S R F S N W I K S N I A N A L K F V K N K L T [1074]
#PAg_2s6_1 CACCGACGGCCGACAACGAAACGAACAAGATCGCGGAGGCGTTCAATCGGATATCGCGCTTCTCCAaCTGGATCAAGTcGAACATCGCCAACGCGCTCAAGTTCGTGAAAAACAAGTTAA [600]
#PAg_2s6_2 .....

      S Q I [1078]
#PAg_2s6_1 CAAGCCAGATTGC [613]
#PAg_2s6_2 .....

```

Underline = primers for sequencing
Squares = common *kdr* sites

Insecticide resistance and underlying targets-site and metabolic mechanisms in *Aedes aegypti* and *Aedes albopictus* from Lahore, Pakistan

Rafi Ur Rahman, Barbara Souza, Iftikhar Uddin, Luana Carrara, Luiz Paulo Brito, Monique Melo Costa, Muhammad Asif Mahmood, Sozaina Khan, Jose Bento Pereira Lima, Ademir Jesus Martins

Aedes aegypti - Nav gene - IIIS6 segment
Pakistani population - IIIS6 haplotypes

```

                                     1520:T/I
S R E V S Y C E I E L V T N D L L T I L R P R S F Q V G K Q P I R E T N I Y M Y
#PAg_3s6_1 TCGCGGGAGGTAAGTTATTGTGAAATCGAACTTGTTACGAATGATCTGCTTACAATTTTACGTCTCGATCCTCCAGGTGGGAAAGCAGCCGATTTCGCGAGACC AACATCTACATGTAC [120]
#PAg_3s6_2 .....
#PAg_3s6_3 .....T.....

                                     1534:F/C
L Y F V F F I I F G S F F T L N L F I G V I I D N F N E Q K K K A G G S L E M F
#PAg_3s6_1 CTCTACTTTGTGTTCTTCATCATCTTCGGGTCGTTCTTCACGCTGAATCTGTTTCATCGGTGTCATCATCGACAACCTCAACGAGCAGAAGAAGAAAGCCGGTGGCTCACTGGAAATGTTC [240]
#PAg_3s6_2 .....G.....
#PAg_3s6_3 .....G.....

M T E D Q K K Y Y N A M K K M G S K K P L K A I P R P R
#PAg_3s6_1 ATGACGGAGGATCAGAAAAAGTACTACAACGCCATGAAAAAGATGGGCTCGAAGAAGCCGCTGAAAGCTATTCCACGGCCTAGGtaaggcatttccatcgcacatcaac [350]
#PAg_3s6_2 .....
#PAg_3s6_3 .....

```

Similar sequences on GenBank

| Haplotype | GenBank accession number |
|-----------|--|
| PAg_3s6-1 | MF794973, MF794979, MF794985, KM677248, KM677249, KM677255, KM677256, KM677258, KM677249, KY078303, MN413379, KF527415, KY046223, KY046225, KY046228, KY046229, KY046231, KY046237, KP861899, KM519597, MN602782. |
| PAg_3s6-2 | MF794983, MF794989, KM677247, KM677253, KM677278, KM677267, KM677268, KM677266, KM677264, KM677273, KM677276, KM677263, MK005557, MK005556, MK005562, MK005567, MK005563, MK005564, MK005565, MK005566, MK005567, KY078304, MK495873, KF527414, KY046224, KY046232, KY046236, KP861896, MN602780 |
| PAg_3s6-3 | MF794977, KM677275, MN602779 |

Insecticide resistance and underlying targets-site and metabolic mechanisms in *Aedes aegypti* and *Aedes albopictus* from Lahore, Pakistan

Rafi Ur Rahman, Barbara Souza, Iftikhar Uddin, Luana Carrara, Luiz Paulo Brito, Monique Melo Costa, Muhammad Asif Mahmood, Sozaina Khan, Jose Bento Pereira Lima, Ademir Jesus Martins

B - *Aedes albopictus*

Aedes albopictus - *Nav* gene - IIS6 segment, exons 20-21

Pakistani population - IIS6 haplotypes

882:L/M

N V D R F P D K D L P R W N F T D F M H S F M I V F R V L C G E W I E S M W D C

```
#PAb_2s6-1 ACAATGTGGATCGCTTCCCGGACAAGGACCTGCCGCGGTGGAACCTCACCGACTTCATGCACCTCGTTCATGATCGTGTCCGGGTGCTGTGCGGCGAGTGGATCGAATCCATGTGGGACT [120]
#PAb_2s6-2 .....
#PAb_2s6-3 .....
#PAb_2s6-4 .....R.....
#PAb_2s6-5 .....Y.....
```

M L V G D V S C I P F F L A T V V I G N L V

```
#PAb_2s6-1 GCATGTGGTTCGGCGACGTGTCTGTATTCCGTTCTTTTGGCCACCGTAGTGATAGGAAATCTAGTAGtaagtatcctctttcgtccagaagtgctgctgacctgacgaacgagtagtacta [240]
#PAb_2s6-2 .Y.....-----.....ta
#PAb_2s6-3 .....Y.....-----
#PAb_2s6-4 .T.....-----
#PAb_2s6-5 .Y.....sy.....k.yymrrmrkksykskkkrn.n.msr.ww.....
```

V L N L F L A L L L S N F G S S S L S A P T A D N E T N K I A

```
#PAb_2s6-1 atcggagaatgcttttcccccaatccagGTACTTAACCTTTTCTTAGCCTTGCTTTGTCCAATTCGGTTCATCGTTCGCTGTCGGCACCGACGCGCCGACAACGAAACGAACAAGATCG [360]
#PAb_2s6-2 .....Y.....Y.....Y.....
#PAb_2s6-3 -----
#PAb_2s6-4 -----
#PAb_2s6-5 .....w.y.....
```

E A F N R I S R F S N W I K S N I A N A L K F V K N K L T S Q I

```
#PAb_2s6-1 CGGAGGCGTTCAACCGGATATCGCGCTTCTCCAACCTGGATCAAGTCCAACATCGCCAACGCGCTCAAGTTCGTGAAAAACAAGTTAACAAGCCAGATTGC [460]
#PAb_2s6-2 .....
#PAb_2s6-3 -----
#PAb_2s6-4 -----
#PAb_2s6-5 .....

```

Similar sequences on GenBank

| Haplotype | GenBank accession number |
|-----------|--|
| PAb_2s6-2 | MK201618/ MK201609, MK201615, MK201617 |
| PAb_2s6-4 | KX281169, KX281170, FJ479615, MK201602/ MK201609, MK201615, MK201617 |
| 882 TTG | MF776970 |

Insecticide resistance and underlying targets-site and metabolic mechanisms in *Aedes aegypti* and *Aedes albopictus* from Lahore, Pakistan

Rafi Ur Rahman, Barbara Souza, Iftikhar Uddin, Luana Carrara, Luiz Paulo Brito, Monique Melo Costa, Muhammad Asif Mahmood, Sozaina Khan, Jose Bento Pereira Lima, Ademir Jesus Martins

Aedes albopictus - *Nav* gene - IIIS6 segment
Pakistani population - IIIS6 haplotypes

```

      Q V G K Q P I R E T N I Y M Y L Y F V F F I I F G S F F T L N L F I G V I I D N
#Pab_3s6-1 CAGGTGGGCAAGCAGCCGATTTCGCGAGACCAACATCTACATGTACCTCTACTTTGTGTTCTTCATCATCTTCGGGTCGTTCTTCACCCTCAACCTGTTTCATCGGTGTCATCATCGACAAC [120]
#Pab_3s6-2 .....C.....
#Pab_3s6-3 .....
#Pab_3s6-4 .....
#Pab_3s6-5 .....Y.....
#Pab_3s6-6 .....R.....Y.....Y..Y.....
#Pab_3s6-7 .....R.....Y.....Y..Y.....
#Pab_3s6-8 .....R.....Y.....C.....Y..Y.....

```

```

      F N E Q K K K A G G S L E M F M T E D Q K K Y Y N A M K K M G S K K P L K A I P
#Pab_3s6-1 TTCAACGAGCAGAAGAAGAAAGCCGGTGGCTCGCTGGAAATGTTTCATGACGGAGGATCAGAAAAAGTACTACAACGCAATGAAAAAGATGGGCTCGAAGAAGCCGCTGAAAGCTATCCCT [240]
#Pab_3s6-2 .....
#Pab_3s6-3 .....A.....
#Pab_3s6-4 .....R.....
#Pab_3s6-5 .....R.....
#Pab_3s6-6 .....
#Pab_3s6-7 .....R.....
#Pab_3s6-8 .....

```

```

      R P R V R
#Pab_3s6-1 CGGCCTAGGGTAAGG [255]
#Pab_3s6-2 .....
#Pab_3s6-3 .....
#Pab_3s6-4 .....
#Pab_3s6-5 .....
#Pab_3s6-6 .....
#Pab_3s6-7 .....
#Pab_3s6-8 .....

```

Similar sequences on GenBank

| Haplotype | GenBank accession number |
|-----------|--------------------------|
| PAb_3s6-1 | MN433760 |
| PAb_3s6-2 | KC1552046, MN433786 |

Insecticide resistance and underlying targets-site and metabolic mechanisms in *Aedes aegypti* and *Aedes albopictus* from Lahore, Pakistan

Rafi Ur Rahman, Barbara Souza, Iftikhar Uddin, Luana Carrara, Luiz Paulo Brito, Monique Melo Costa, Muhammad Asif Mahmood, Sozaina Khan, Jose Bento Pereira Lima, Ademir Jesus Martins

Alignment of IIS6 and IIS6 segments haplotypes of the voltage gated sodium channel gene of *Aedes aegypti* and *Aedes albopictus* from Lahore, Pakistan. Nucleotides in the exon and intron regions are in upper- and lowercase letters, respectively. Dots indicate the same nucleotides as the haplotype in the first row. Non-synonymous SNPs changes are in red. The amino acid translation is indicated above the alignment in blue, numbered according to the Na_v protein of *Musca domestica*, as reference. The regions of primers alignment are underlined.