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## Research Article

# Detection of Voltage-Gated Sodium Channel (VGSC) Mutation in Deltamethrin-Resistant *Blattella germanica* L. from Indonesia

<sup>1</sup>Resti Rahayu, <sup>2</sup>Hasmiwati and <sup>1</sup>Muhammad Rizki Saputra

<sup>1</sup>Department of Biology, Faculty of Mathematics and Natural Sciences Andalas University, Padang 25163, West Sumatera, Indonesia

<sup>2</sup>Department of Parasitology, Faculty of Medicine Andalas University, Padang 25129, West Sumatera, Indonesia

## Abstract

**Background and Objective:** German cockroach (*Blattella germanica* L.) is one of the most common residential pests in Indonesia. Controlling the population face obstacles due to insecticide resistance, especially to deltamethrin. This research investigated the resistance status and the possibility of a Voltage-Gated Sodium Channel (VGSC) knockdown resistance mutation (L1014F) in two field strains of German cockroaches collected from two cities in Indonesia (Bukittinggi, named RMKN-BKT and Bandung, named KRSA-BDG) with VCRU-WHO as the standard strain. **Materials and Methods:** This study started with a bioassay test to determine the lethal dose of 50% (LD<sub>50</sub>) for each strain and followed by a molecular test for mutation detection. **Results:** The results showed that the RMKN-BKT and KRSA-BDG strains were highly resistant to deltamethrin with RR50 values of 80,090 times and 73,272 times, respectively. Only the RMKN-BKT strain was shown to carry L1014F kdr mutation which lead to an amino acid replacement from leucine (TTG) to phenylalanine (TTC). Two silent mutations were also found in both field strains at codons 983 (TGC/cysteine → TGT/cysteine) and 984 (GGG/glycine → GGA/glycine) which were suggested as polymorphism phenomena. The absence of the L1014F mutation in the Bandung strain does not exclude the possibility of the presence of the VGSC mutation at other points. **Conclusion:** It requires subsequent investigation in mutation detection at other points and the possible presence of other resistance mechanisms to get a precise solution in the population control. Bioinsecticides may stand as a breakthrough so that the strategy will no longer focus on insecticides.

**Key words:** German cockroach, voltage-gated sodium channel, gene mutation, residential pests, resistance mechanism, deltamethrin, knockdown resistance

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**Corresponding Author:** Resti Rahayu, Department of Biology, Faculty of Mathematics and Natural Sciences andalas University, Padang 25163, West Sumatera, Indonesia

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**Competing Interest:** The authors have declared that no competing interest exists.

**Data Availability:** All relevant data are within the paper and its supporting information files.

## INTRODUCTION

German cockroach (*Blattella germanica* L.) is included in the six major urban pests<sup>1</sup>. They are detrimental both economically and medically<sup>2,3</sup> as well as vector agents of various pathogens for humans<sup>4</sup>. A study showed that the average global infestation of *B. germanica* in human habitation ranged from 40 to 70%<sup>5</sup>. Insecticides are still involved in controlling this pest. However, the intensive use has given rise to insecticide resistance and led to control failure<sup>6</sup>. The application of insecticides has become a common practice in many countries, including in Indonesia where deltamethrin is popularly applied. Deltamethrin is a synthetic pyrethroid insecticide classified as contact and digestive poison<sup>7</sup>, targetting the Voltage-Gated Sodium Channel (VGSC) within the neural cells of insects.

One of the best-known mechanisms conferring resistance to insecticides is target site modification<sup>8</sup>. In *B. germanica*, previous studies have reported various mutations in the VGSC gene, among them are D58G, P1880L<sup>9</sup>, E434K, C764R<sup>9,10</sup> and L1014F (L993F)<sup>9-13</sup> which induce the amino acids substitutions and an increase in insecticide resistance to pyrethroid. The cockroach population from two cities: Bukittinggi (RMKN-BKT) and Bandung (KRSA-BDG) has been reported to be progressively difficult to control despite regular mitigation efforts applied. It is suspected that both strains have developed resistance to pyrethroid insecticides. The sampling sites where both strains originated are known as tourism destinations with high mobilized communities and insecticides are common in use to control the cockroaches. Therefore, this study aims to determine the resistance status of the two strains (RMKN-BKT and KRSA-BDG) and explore the VGSC mutation that is probably present in these field strains, particularly the L1014F mutation, the most common VGSC mutation in *B. germanica*.

## MATERIALS AND METHODS

**Study area:** This study was conducted in February to June, 2018 at the Laboratory of Animal Physiology, Faculty of Mathematics and Natural Sciences and the Biomedical Laboratory, Faculty of Medicine Andalas University.

**Insects and rearing:** The field strain German cockroaches were obtained from two cities in Indonesia, Bukittinggi (called RMKN-BKT collected from restaurants) and Bandung (named KRSA-BDG collected from restaurants) (Fig. 1). While the standard strain came from the Vector Control Research Unit (VCRU-WHO), School of Biological Sciences, University of Science Malaysia (USM), Penang, Malaysia. The strains were

maintained in the laboratory at room temperature (25-28°C), 80-86% relative humidity and photoperiod 12:12. They were fed dog food (Pedigree®) while water was provided *ad libitum*. In total, 750 insects were used for the bioassay test specified as German male cockroaches aged between 1 to 2 months<sup>14</sup>.

**Bioassay test:** A bioassay test<sup>6</sup> was performed to determine the 50% lethal dose (LD<sub>50</sub>) of deltamethrin for each test population. The insecticide deltamethrin 98% was dissolved using acetone, where the initial concentration was 0.03% which was then diluted according to the test concentration used subsequently. To observe the mortality of 5-95% of the population, five concentrations in the range of 0.003-0.03% (0.3-3 µg/insect) were used among the VCRU-WHO standard strain and another five concentrations between 0.003-5% (0.3-500 µg/insect) for field strains (RMKN-BKT and KRSA-BDG). Ten insects were used for each treatment. After the cockroaches are lightly anaesthetized with CO<sub>2</sub>, an insecticide solution is applied topically<sup>6,14</sup>. The insecticide was applied by topical application on the ventral mesothorax of the cockroach. Subsequently, the cockroaches were placed into a 1 L plastic container equipped with wet cotton and food sources (grouping based on the test concentration). The mortality of cockroaches was observed 48 hrs later and the assay was repeated 5 times for each concentration.

## DNA extraction, PCR amplification, sequencing and sequence analysis:

The cockroaches used were individuals from field strains that survived after the previous insecticide treatment. DNA isolation was performed using PureLink Genomic DNA Mini kit from Invitrogen, USA. The Polymerase Chain Reaction (PCR) procedure was used to amplify the partial VGSC sequence of *B. germanica* L., containing codon 1014 using KUF and KUR primers<sup>12</sup> (Table 1). The amplified DNA yields lengths of up to 207 bps. Amplification was performed under the following conditions: Denaturation step at 95°C for 1 min, 45 cycles of amplification (15 sec at 95°C, 15 sec at 60°C and 10 sec at 72°C) and an extension step at 72°C for 5 min. The samples amplified by PCR were subsequently stored at -20°C. PCR products were then visualized by gel electrophoresis on a 1.5% agarose gel at 100 V for 60 min followed by a purification step. Sequencing was undertaken by Macrogen (Seoul, Korea) with subsequent sequence analysis using Geneious Software Version 11.1.2 from Biomatters Ltd., Auckland, New Zealand.

Table 1: Primers used for amplification of the knockdown resistance (kdr) on sodium channel gene in *Blattella germanica* L.

Primer	Sequence
KUF	ATGATTGTGTTCCGAGTGTG
KUR	TCCCTGACCAACCTGTGAAG

**Data analysis:** Dose-response of mortality was analyzed with probit analysis by the Polo-PC program, to determine LD<sub>50</sub>. The resistance status of the tested strain was determined by using the resistance ratio (RR<sub>50</sub>) through a comparison of the LD<sub>50</sub> value between the tested field strain and the standard strain. The category of resistance ratio (RR<sub>50</sub>) is  $\leq 1$  = not resistant,  $>1-\leq 5$  = low resistance,  $>5-\leq 10$  = moderate resistance,  $>10-\leq 50$  = high resistance,  $>50$  = very high resistance<sup>15</sup>. A VGSC DNA and amino acid sequence of a German cockroach that mutated (accession number KC731438.1) on L1014F was used as representative for sequence analysis. The sequencing output was analyzed using Geneious Software version 11.1.2 to detect any mutation in the VGSC sequence.

## RESULTS

**Resistance status of German cockroach to deltamethrin:** The result of the resistance status of German cockroaches from each strain was given in Table 2. It showed that the two field strains (RMKN-BKT and KRSA-BDG) have been highly resistant to deltamethrin.

**VGSC sequence analysis in RMKN-BKT and KRSA-BDG strains:** The VGSC sequence comparison on RMKN-BKT and KRSA-BDG strains were shown in Fig. 2. The VGSC sequence is detected from nucleotide 2947-3153 or C983-G1051. Figure 2 outlines the mutation on the RMKN-BKT strain observed in

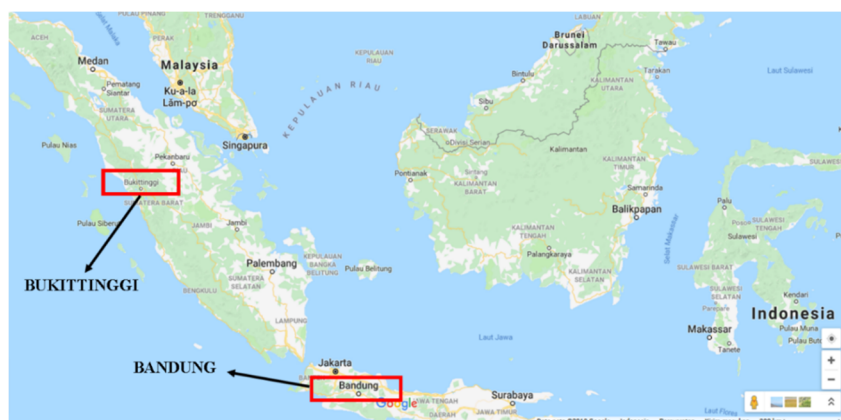


Fig. 1: Sampling location for two strains of German cockroaches  
RMKN-BKT (Bukittinggi) and KRSA-BDG (Bandung) (Source: Google Maps 2019)

KC731438.1	1	TGCGGGGAGTGGATAGAGTCTATGTGGGATTGTATGCTTGTGGAGAC	
RMKN-BKT	1	TGTGGAGAGTGGATAGAGTCTATGTGGGATTGTATGCTTGTGGAGAC	
KRSA-BDG	1	TGTGGAGAGTGGATAGAGTCTATGTGGGATTGTATGCTTGTGGAGAC	
Amino acid	1	C G E W I E S M W D C M L V G D	
		a b	
	17	TGGTCCTGCATCCCGTTCTTCTTGGCCACTGTCGTCATTGGAACCTTC	
	17	TGGTCCTGCATCCCGTTCTTCTTGGCCACTGTCGTCATTGGAACCTTC	
	17	TGGTCCTGCATCCCGTTCTTCTTGGCCACTGTCGTCATTGGAACCTTC	
	17	W S C I P F F L A T V V I G N	
		c	
	33	GTTGTAAGTAGAAATAATTGTAACCATCTGAATTGTCCTTTTTTTTAA	
	33	GTTGTAAGTAGAAATAATTGTAACCATCTGAATTGTCCTTTTTTTTAA	
	33	GTTGTAAGTAGAAATAATTGTAACCATCTGAATTGTCCTTTTTTTTAA	
	33	V V S R N N C N H L N C P F F Stop	
	49	ATTTTATTTATGTATTTGAGTGAACCAGAAATCTATCAAAAATCT	63
	49	ATTTTATTTATGTATTTGAGTGAACCAGAAATCTATCAAAAATCT	63
	49	ATTTTATTTATGTATTTGAGTGAACCAGAAATCTATCAAAAATCT	63
	49	I F F M Y F S E P E I Y Q K S	

Fig. 2: Sequence analysis of VGSC gene in two field strains of *Blattella germanica* L. (Bg) (GenBank: KC731438.1)  
a,b: Two silent mutations in both field strains [codon 983 (TGC/cysteine→TGT/cysteine) and codon 984 (GGG/glycine→GGA/glycine)] and c: Point mutation (L1014F) in RMKN-BKT strain (TTG/leucine→TTC/phenylalanine)

Table 2: Resistance status of German cockroach (*Blattella germanica* L.) to deltamethrin in tested strains

Population	N	LD <sub>50</sub> (µg/insect)	Slope±SE	RR <sub>50</sub>	Category of resistance ratio
VCRU-WHO/S	250	0.022	0.965±0.162	1	Not resistant
RMKN-BKT/R	250	1.762	0.579±0.109	80.090	Very high resistance
KRSA-BDG/R	250	1.612	0.579±0.113	73.272	Very high resistance

S: Vulnerable strain/standard strain, R: Field population, N: Number of German cockroach tested, LD<sub>50</sub>: Lethal doses to kill 50% of the population, observed on 48 hrs after treatment and RR<sub>50</sub>: Resistance ratio (LD<sub>50</sub> R per LD<sub>50</sub> S)

codon 1014, imposing the change from leucine into phenylalanine (L1014F). Meanwhile, mutations/polymorphism were observed in both strains (RMKN-BKT and KRSA-BDG) at C983 (TGC→TGT) and G984 (GGG→GGA) with no alteration in amino acid.

## DISCUSSION

This study confirmed that both field strains (RMKN-BKT and KRSA-BDG) have developed resistance to deltamethrin. The resistance rate in the RMKN-BKT and KRSA-BDG was more than 80 and 73 times, respectively. In *B. germanica*, deltamethrin-resistant cases have been reported previously in several countries such as Malaysia<sup>16</sup>, Singapore<sup>17</sup>, America<sup>12</sup>, Nigeria<sup>18</sup>, South Korea<sup>19</sup> and Argentina<sup>20</sup>. The widespread of deltamethrin-resistant arises presumably due to insecticides' overuse and/or misuse. It is necessary to re-evaluate the insecticides used and take the alternative tactics to control the cockroaches to suppress the development of resistance.

Sequencing analysis showed that the RMKN-BKT strain possesses L1014F mutation (TTG/leucine→TTC/phenylalanine). This mutation is located in domain II and transmembrane segment 6 (S6) on exon 20 VGSC. The structure of VGSC is a transmembrane protein present in neurons and regulates the action potential of cells<sup>21</sup>. VGSC is the target site of deltamethrin. In consequence, any changes due to gene mutations will cause target site insensitivity resulting in insecticide resistance<sup>22</sup>. The previous studies have represented that the L1014F mutation was well-known as a pyrethroid-resistance-associated mutation and it has been widely presented in the German cockroach<sup>9-13</sup>.

Despite having a high level of resistance, the contrary, L1014F mutation was absent in the KRSA-BDG strain. We suggest that the target site mutation in KRSA-BDG probably takes place elsewhere in the different codons or other resistance mechanisms have been implicated so that this strain becomes resistant to deltamethrin. In *B. germanica*, it is known that there are three main resistance mechanisms: Metabolic detoxification, physiological resistance and behavioural resistance<sup>1</sup>. The absence of the L1014F mutation in the KRSA-BDG strain implies the existence of target-site mutations at another point and/or the availability of other resistance mechanisms in that strain leading to resistance to deltamethrin. Due to the high resistance level, it is strongly

suggested that more than one mechanism exists in both the RMKN-BKT and KRSA-BDG strains. The presence of those resistance mechanisms together ultimately strengthens the resistance phenotype in those strains.

Further, there were two silent mutations at points C983 and G984 in both strains. Mutation at C983 (TGC→TGT) encodes the amino acid cysteine while mutation at G984 (GGG→GGA) encodes amino acid glycine. These mutations are known as polymorphism. Even though it seems trivial, the current discovery in the genetic field has supported that a silent mutation in eukaryotes could affect specialized processes in gene-splicing and gene expression<sup>23,24</sup>. Although, there is no alteration in the protein sequence, the DNA and mRNA sequences are altered due to the silent mutation<sup>24</sup>.

A high level of resistance in both field strains signals the need to rotate the insecticides used in controlling the cockroaches, particularly for both cities. This rotation should involve the insecticides that do not target the VGSC site, such as carbamate and organophosphate. Insecticide resistance monitoring is also required aside from the enforcement of the control strategy of the cockroaches population. Alternatively, controlling German cockroaches could be more eco-friendly through the application of bio-insecticide such as *Cymbopogon flexuosus*<sup>25,26</sup>, *C. nardus*<sup>27</sup>, pepper *Scinus molle*<sup>28</sup>, *Carica papaya*<sup>29</sup> and *Morinda citrifolia* L.<sup>30</sup>.

The presence of L1014F mutation has confirmed the existence of the target-site mechanism in RMKN-BKT. However, other mutations in VGSC perhaps also take place in KRSA-BDG as well as in RMKN-BKT. To prevent control failure, this study requires subsequent thorough exploration at the molecular level together with other resistance mechanisms that probably contribute to the resistance in those two German cockroach strains to deltamethrin. The comprehension of existing mechanisms most likely generate an effective strategy in controlling cockroach populations, primarily for both cities known as tourist city.

## CONCLUSION

Our study showed that the two field strains of *B. germanica* from two cities in Indonesia (RMKN-BKT and KRSA-BDG) possess a high level of resistance to deltamethrin. A pyrethroid-resistance-associated mutation in the VGSC gene, L1014F, was present in RMKN-BKT however not in KRSA-

DG. The absence of L1014F mutation in KRSA-BDG and a high level of resistance in both strains suggest the presence of other mutations and/or another resistance mechanism that contribute to the resistance phenotype of the two strains. Moreover, two silent mutations in the VGSC gene were detected at codon C983 (TGC/cysteine→TGT/cysteine) and G984 (GGG/glycine→GGA/glycine) in both strains. The application of bioinsecticides could be an alternative option in controlling the German cockroach population.

### SIGNIFICANCE STATEMENT

This research explored the Voltage-Gated Sodium Channel (VGSC) knockdown resistance mutation (L1014F) in two field strains of German cockroaches (*Blattella germanica* L.) collected from two cities in Indonesia (RMKN-BKT and KRSA-BDG). Sequence analysis showed that the L1014F kdr mutation was detected only in RMKN-BKT strains (TTG/leucine→TTC/phenylalanine). Besides, two silent mutations were found in both field strains at codons 983 (TGC/cysteine→TGT/cysteine) and 984 (GGG/glycine→GGA/glycine). Another resistance mechanism may take place in those strains. Vector control management in the community requires alternative ways with no insecticides implicated.

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