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BRIEF COMMUNICATION

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Monitoring of Pyrethroid Resistance Allele Frequency in the Common Bed Bug (*Cimex lectularius*) in the Republic of Korea

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Abstract: Two-point mutations (V419L and L925I) on the voltage-sensitive sodium channel of bed bugs (*Cimex lectularius*) are known to confer pyrethroid resistance. To determine the status of pyrethroid resistance in bed bugs in Korea, resistance allele frequencies of bed bug strains collected from several US military installations in Korea and Mokpo, Jeollanam-do, from 2009-2019 were monitored using a quantitative sequencing. Most bed bugs were determined to have both of the point mutations except a few specimens, collected in 2009, 2012 and 2014, having only a single point mutation (L925I). No susceptible allele was observed in any of the bed bugs examined, suggesting that pyrethroid resistance in bed bug populations in Korea has reached a serious level. Large scale monitoring is required to increase our knowledge on the distribution and prevalence of pyrethroid resistance in bed bug populations in Korea. Based on present study, it is urgent to restrict the use of pyrethroids and to introduce effective alternative insecticides. A nation-wide monitoring program to determine the pyrethroid resistance level in bed bugs and to select alternative insecticides should be implemented.

Key words: *Cimex lectularius*, bed bug, insecticide resistance, pyrethroids, quantitative sequencing, voltage-sensitive sodium channel

The common bed bug, *Cimex lectularius* L. (Hemiptera: Cimicidae), is an blood feeding ectoparasite associated with humans, but also feeds on alternate domestic hosts [1-3]. Bed bugs are widely distributed in temperate and subtropical regions, especially in developing nations, where they are considered a serious indoor nuisance [4]. Although bed bugs are not considered a disease vector, they can mechanically transmit hepatitis B virus [5] and human immunodeficiency virus [6].

Introduction of synthetic insecticides after World War II greatly reduced bed bug infestations among human habitations [7]. However, the incidence of bed bug infestations has dramatically increased in the United States (US), the United Kingdom and other countries in recent years [4]. Increased international travel, trade and immigration have undoubtedly contributed to the widespread introduction of bed bugs and their prevalence among households, hotels, and other places where people congregate [4]. Several cases of domestic bed bug infestations have been also reported in Korea [8].

Insecticides are the most effective means of bed bug control. Pyrethrin and synthetic pyrethroids (e.g., deltamethrin, cyhalothrin and permethrin) are extensively used for bed bug control in residences, hotels and commercial operations [9]. Unfortunately, the bed bug control practice almost exclusively using pyrethroids has resulted in high levels of resistance to this class of insecticides in bed bugs. A field-collected population of bed bugs in the United Kingdom was determined to be resistant to pyrethroids and carbamates [10]. Later, bed bug populations resistant to deltamethrin and λ-cyhalothrin were reported in the United States [11,12].

Molecular analysis of deltamethrin resistance in a bed bug population revealed that 2 point mutations (V419L and L925I) in the voltage-sensitive sodium channel (VSSC) α-subunit gene (*Clvssc*) were primarily responsible for resistance to deltamethrin, whereas metabolic factors did not seem to be involved [13]. A quantitative sequencing (QS) protocol was established as a population-based genotyping tool and

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used as a molecular resistance monitoring tool to determine the frequency of the 2 mutations in bed bug populations [14].

In this study, the QS protocol was employed to predict the frequencies of 2 mutations in bed bugs collected from several US military installations and a Korean residence in Mokpo, Jeollanam-do in Korea from 2009-2019.

Nymphs and adult bed bugs were collected from Camp Red Cloud, Uijeongbu, Gyeonggi-do (2009 and 2013), Camp Walker, Daegu (2009 and 2012), Yongsan Dragon Hill Hotel, Seoul (2010), Yongsan Garrison, Seoul (2010, 2016, and 2017), Camp Casey, Dongducheon, Gyeonggi-do (2011 and 2013), Osan Air Base, Osan, Gyeonggi-do (2013-2015) and Camp Humphreys, Pyeongtaek, Gyeonggi-do (2018-2019), and stored in 95% ethyl alcohol (EtOH) at -20˚C until used for DNA extraction. Bed bugs were also collected from a residence in Mokpo, Jeollanam-do, in 2019 and immediately stored at -20˚C until used. The locations of bed bug collections are shown in Fig. 1.

The bed bug specimens stored in EtOH were incubated in TE buffer at 4˚C for 10-15 hr to remove residual EtOH before gDNA extraction. Bed bugs (1-7 individuals; numbers vary by region and developmental stage) were transferred into liquid nitrogen for snap freezing and pulverized with mortar and pestle. gDNA was extracted using DNesay Blood & Tissue Kit

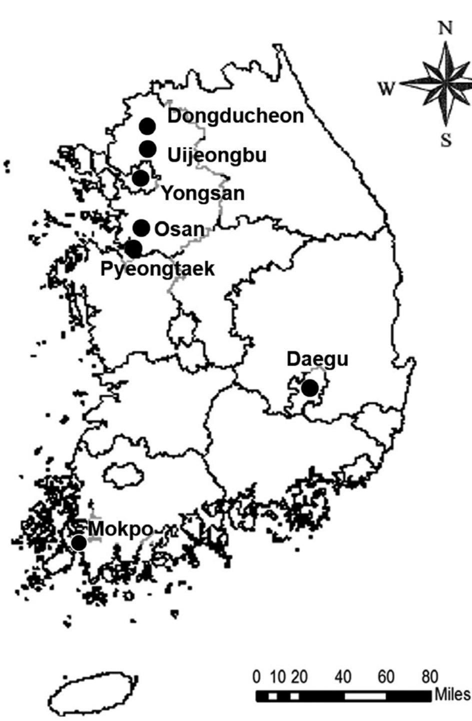

Fig. 1. Bed bug collection sites.

(Qiagen, Valencia, California, USA) according to the manufacturer's instructions. Each gDNA was diluted to 5 ng/µl and used as template for PCR amplification. To amplify the 2 Clvssc gene fragments, each encompassing V419L and L925I mutation site, PCR was conducted in 25 µl of PCR reaction mixture containing 4 µl of template DNA (5 ng/µl), 1 µl of forward and reverse primers (5 pmole/µl each) and 0.12 µl Ex Taq (Takara, Shiga, Japan). PCR thermal cycling was performed for 35 cycles of 95˚C for 30 sec, 61˚C for 30 sec and 72˚C for 1 min. All primers were based on Seong et al. [14], except for a primer used for sequencing (5′QS-LI; 5′-CAGCTCAGGGTGTTTAAGCT-3′) to increase the accuracy of QS.

QS was conducted according to Seong et al. [14]. The equations for the resistance allele frequency prediction were: $y=110.0\times-1.52$ ($r^2=0.9928$) and $y=119.6\times+0.59$ ($r^2=0.9997$) for the V419L or L925I mutations, respectively (x = resistance allele signal, y = resistance allele frequency) [14].

QS results revealed that the L925I mutation was present in all beg bugs sampled from US military installations and a Korean residence. The V419L mutation was also observed in all bed bugs sampled, except a few individual bed bugs collected in 2009, 2012, and 2014. This finding suggests that considerable levels of pyrethroid resistance have developed in bed bug populations in Korea, especially US military installations (Table 1).

The V419L mutation was not observed in some individual bed bugs collected in 2009, 2012, and 2014 (Table 1). However, the L925I mutation frequency was 100%, indicating that the 2 mutations can be present as independent alleles and that there are 2 different resistant haplotypes (one with only the L925I mutation and the other with both V419L and L925I mutations). The V410L mutation was saturated to 100% in bed bugs collected from the same site (Osan air base, Pyeongtaek, Korea) following year (2015), indicating a strong pyrethroid selection pressure at this site. Nevertheless, both mutations appeared to be saturated in all bed bug populations collected in more recent years (2015-2019) (Table 1).

As all material supplies and military dispatches occur directly from the US mainland to US military bases in Korea, the pyrethroid-resistant bed bugs collected from the US military bases in Korea most likely originated from the US or other foreign US bases. However, this does not preclude the possibility of military members acquiring bed bugs at other residences in Korea. The current status of saturated resistance allele frequencies strongly suggests that pyrethroid resistance is widespread in bed bug populations at US military bases examined, thus

Table 1. Pyrethroid resistance allele frequency in regional populations of bed bugs

Collection site	Collection date	superscript No.[a]	superscript Stage[b]	Allele frequency (%)	
				V419L	L925I
Camp Red Cloud, Uijeongbu	2009. 9. 9	5	A	0	100
Camp Walker, Daegu	2009. 9. 14	2	A	0	100
Yongsan Dragon Hill Hotel, Seoul	2010. 7. 2	5	3rd N	100	100
Camp Casey, Dongducheon	2011. 1. 27	5	A	100	100
Camp Walker, Daegu	2012. 10. 25	4	A	0	100
Camp Casey, Dongducheon	2013. 1. 23	1	A(F)	100	100
		2	A(M)	100	100
Osan Air Base, Osan	2013. 10. 18	1	A(F)	100	100
		1	A(M)	100	100
Camp Red Cloud, Uijeongbu	2014. 7. 25	1	A(F)	0	100
Osan Air Base, Osan	2014. 9. 19	1	A(F)	0	100
	2015. 1. 21	5	A(F)	100	100
		5	A(M)	100	100
Yongsan Garrison, Seoul	2016. 2. 10	3	A(F)	100	100
		3	A(M)	100	100
	2016. 11. 7	2	A(F)	100	100
	2017. 2. 8	2	A(M)	100	100
Camp Humphreys, Pyeongtaek	2018. 11. 7	5	A(F)	100	100
		5	A(M)	100	100
	2018. 11. 14	2	A(F)	100	100
		3	A(M)	100	100
	2019. 2. 19	5	A (F)	100	100
		3	A(M)	100	100
	2019. 2. 20	6	A(F)	100	100
		7	A(M)	100	100
	2019. 2. 25	4	A(F)	100	100
		6	A(M)	100	100
	2019. 3. 4	7	A(F)	100	100
		5	A(M)	100	100
Private residence, Mokpo	2019. 9	3	A(F)	100	100

[a]Individual numbers of bed bug used for QS.
[b]Developmental stage of pooled bed bugs for QS. A, adults with sex unidentified; 3rd N, 3rd Nymph; A(F), female adult; A(M), male adult.

the use of pyrethroid insecticides for bed bug control most likely would not be effective, immediately requiring new alternatives. To control pyrethroid-resistant bed bug populations, neonicotinoid (e.g., imidacloprid, acetamiprid and dinotefuran) and pyrazole insecticides (e.g., chlorfenapyr) have already been used as alternative insecticides in the US [15,16]. Thus, application tests for these new alternative insecticides for bed bug control are required in Korea.

Interestingly, 100% resistance allele frequency was also observed in the bed bugs collected from Mokpo, a port city distant from the US military installations (Table 1). This is the first report identifying saturated resistance allele frequencies in domestic bed bugs that were not sampled from the US military installations, indicating that pyrethroid resistance is likely widespread throughout Korea. Since bed bugs have been reported just recently in domestic areas and the use history of pyrethroids for bed bug control is short, the 100% resistance allele frequency in Mokpo bed bugs is not likely due to local selection, but rather due to the introduction of bed bugs from foreign countries, although the origin of the bed bugs was not determined. Considering that the increased trends in international travel and trade likely accelerates the introduction of pests, including bed bugs, a nation-wide surveillance program for the assessment of risk factors of invasive pests, such as the insecticide resistance traits, is urgently needed. As demonstrated in this study, QS can be readily applicable for any bed bugs stored in 95% EtOH, thus serving as an effective tool for nation-wide monitoring of bed bud resistance. When high levels of pyrethroid resistance are found, new insecticides with different modes of action should be applied for the efficient management of pyrethroid-resistant bed bugs. Among the public health insecticides currently registered in Korea, imidacloprid, dinotefuran and chlorfenapyr are primary options for replacing pyrethroids as new bed bug control agents. However, field resistance to any insecticide used for bed bug control should be carefully monitored and reported to ensure whether effective control is achieved.

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CONFLICT OF INTEREST

We declare that there is no conflict of interests in this study.

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