Proceedings of the Eighth International Conference on Urban Pests
Gabi Müller, Reiner Pospischil and William H Robinson (editors) 2014
Printed by OOK-Press Kft., H-8200 Veszprém, Papái ut 37/a, Hungary

INSECTICIDE SUSCEPTIBILITY AND RESISTANCE MECHANISMS IN BODY LICE IN RUSSIA

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Abstract The majority of body lice colonies examined in Moscow were permethrin resistant and susceptible to organophosphorus (fenthion, malathion) and neonicotinoid insecticides. Metabolic resistance of body lice to permethrin was investigated using synergists piperonyl butoxide (PBO), S,S,S-tributylphosphorotrithioate (DEF) and diethylmaleate (DEM). Synergism with enzyme inhibitors in permethrin-resistant body lice demonstrated that in some cases enhanced metabolism was involved in pyrethroid resistance. Resistance to permethrin was partially suppressed by` PBO, DEM and DEF, which suggests possible involvement of monooxygenases, esterases and glutathion-S-transferases in the resistance mechanism. Since the use of synergists did not lead to full suppression of resistance, it appears that lice also posses knockdown resistance (*kdr*). Body lice were studied by real-time PCR to detect *kdr* mutations (T917I and L920F) in the para-orthologous voltage-sensitive sodium channel gene, which are associated with permethrin resistance. The frequency of occurrence of the pyrethroids resistance gene was measured in 153 lice. Of these, 101 (66.0%) turned out to be homozygous resistant, 18 (11.8%) homozygous susceptible, and 34 (22.2%) heterozygous.
Key words *Pediculus humanus,* pyrethroid resistance, synergist, knockdown and metabolic resistance, sodium channel mutations.

INTRODUCTION

Body lice (*Pediculus humanus humanus* L.) is a hematophagous ectoparasite that lives on the body and in the clothing or bedding of its human host. They transmit such human diseases as epidemic typhus, relapsing fever, and trench fever. Body lice infestation is usually found only in persons who have no access to clean clothes or bathing facilities (e.g. the homeless population). In 1990, permethrin was introduced for the control of *P. humanus* in Russia but by 2008 it was found ineffective. Research conducted in 2009-2012 revealed that resistant insects are present in over 90% of lice micropopulations collected on the homeless. Permethrin-resistant lice also show cross-resistance to d-phenothrin and DDT (Lopatina and Eremina, 2011, 2013). Permethrin-resistant body lice also have been found in France (Drali et al., 2012).

The main mechanism of pyrethroid resistance in arthropoda involves lower sensitivity of their nervous system to this group of insecticides because of a mutation in the gene responsible for voltage-sensitive sodium channels. Point mutations in □-subunits of the para-orthologous sodium channel gene of the head and body louse (M815I, T917I, and L920F) are associated with permethrin and DDT resistance (Lee et al., 2003; Gao et al., 2003; Clark, 2010). *Kdr* type resistance may be combined with changed activity of enzyme systems. Pyrethroid resistance, however, was investigated in head lice only. It was demonstrated that permethrin-resistant head lice show the most pronounced increase in

the activity of monooxygenases (MO), but a smaller one in that of esterases (Est) and glutathion-S-transferases (G-S-T) (Hemingway et al., 1999; Piccolo et al., 2000; Bartles et al., 2001; Audino et al., 2005). Only limited information on the resistance mechanism in head lice is available (Drali et al., 2012). The present work is a summary of our insecticide resistance monitoring in body lice from 2009 to 2013. Mutations associated with permethrin resistance in body lice have been detected. Evidence of possible involvement of monooxygenases, esterases, and glutathion-S-transferases was also obtained in the course of synergism studies.

MATERIALS AND METHODS

Body lice colonies. Body lice were collected from the clothing of homeless persons in two shelters in Moscow, Russia. The homeless were given new clothes, and their lice-infested clothes were delivered to the laboratory. Clothes of about 1500 homeless persons were examined. Isolated lice samples were also taken in St. Petersburg and Tambov. A susceptible strain of human body lice maintained on rabbits and never exposed to permethrin was used as control. Toxicological experiments were performed only on lice from large micropopulations (samples from single hosts were from 500 to over 6000 insects).

Chemicals. Technical grade insecticides used for baseline susceptibility tests and synergistic studies were permethrin (93.0%), DDT (76.0%) and malathion (92.8%). In the investigation of lice enzyme system technical grades synergists were also used. These were piperonyl butoxide (PBO) (99.0%), a monooxygenases inhibitor, S,S,S-tributylphosphorotrithioate (DEF) (98.3%), an esterase inhibitor, and diethylmaleate (DEM) (100%), a glutathion-S-transferases inhibitor. All technical grade insecticides were diluted in analytical grade acetone. Emulsifiable concentrates of insecticides were diluted in water. These were permethrin (20%), d-phenothrin (10%), malathion (57%), fenthion (20%), and a water soluble concentrate of imidacloprid (20%).

Bioassays. The percentage of resistant lice was established by the method proposed by B.C. Zeichner (Zeichner, 1999) that consisted of placing insects on filter paper impregnated with insecticides (permethrin 0.02 mg cm^{-2}, DDT 1.0 mg cm^{-2}, malathion 0.2 mg cm^{-2}). Knockdown rate was established after six hours of exposure. The sensitivity of older larvae and mature lice to water insecticide emulsion was evaluated by dipping lice into emulsion for 15 minutes with subsequent rinsing in fresh running water. Lice mortality was assessed after 24 hours. For synergism studies, lice were sprayed with synergist solution in acetone (PBO – 0.1%, DEF – 0.1%, DEM 1.0% a.i.) for 30 min. prior to permethrin susceptibility test by a 15 min. immersion in an aqueous emulsion of insecticide. Each experiment was replicated three times.

Genetic Methods. Body lice were studied by real-time PCR to detect the *kdr* mutations (T917I and L920F) in the para-orthologous voltage-sensitive sodium channel gene, which are associated with permethrin resistance. Insects stored prior to DNA extraction in 95% ethanol. Genomic DNA was extracted from individual specimens, using the AmpliSens Riboprep Kit (Central Institute of Epidemiology, Moscow, Russia) according to manufacturer's instructions. The *P.humanus VSSC* – specific forward and reverse primers were, at 360 nmol/L, Ped-F TGG GTC GAA CTG TTG GAG CTT and Ped-R CCA TAA CGG CAA ATA TGA ATA TGA T, respectively. The corresponding dye-labeled probes (final concentration 100 nmol/L) were Ped-S FAM-TGG GTA ATT TAA CAT TCG TCC TTT GCC-BQH1R6G-CCTGGGGA and Ped-R R6G-TGG GTA ATT TAA TAT TCG TCT TTT GCC-BQH1. The PCR conditions were 95°C for 15 min followed by 5 cycles at 95°C for 10 sec., 60°C for 25 sec., and 72°C for 15 sec.; then by 40 cycles at 95°C for 10 sec., 56°C for 25 sec., and

72°C for 15 sec. (Rotor-Gene 6000, Qiagen). The fluorescence signal was recorded at the 56°C step for the last 40 cycles. Each run included negative control and positive recombinant control DNA of the *P. humanus* VSSC *kdr*-resistant and *kdr*-sensitive alleles of gene fragment as a standard. Absence of false-positive PCR results was confirmed direct sequencing of two genomic regions of the sodium channel α-subunit (exon1 and exon3) with primers 5'QSMI and 3'QSTILF (Kwon et al., 2008).

Data Analysis. Data were pooled and subjected to probit analysis using software program (Probit Analysis, v.1.0). Resistance ratio (RR) was calculated as the relation the CL_{50} value of the resistant strain to the same of the susceptible laboratory body lice colony. Synergistic ratio (SR) was calculated as the relation the CL_{50} value for permethrin to CL_{50} value for the synergist and permethrin combination.

RESULTS AND DISCUSSION

Body lice were found in 30% of clothing from homeless persons. Similar figures were obtained in other countries. In Marseille, for instance, body lice were found in 22% of the homeless (Brouqui et al., 2005).

Lice from various locations (Moscow, St. Petersburg, Tambov) were resistant to pyrethroids and DDT, and sensitive to malathion. Generally permethrin-resistant lice comprised 40-60% of the sample while the average was about 45%. Samples of lice with 100% susceptibility to permethrin are rare, and their share dropped during the observation period. In Moscow it was 9.1% in 2009, 3.3% in 2010, 5.4% in 2011, 1.9% in 2012, and none in 2013.

In 2012 studies conducted in St. Petersburg the situation was similar to that in Moscow. Permethrin-resistant lice were found in all samples from St. Petersburg, and their share was about 60%. In 2013 permethrin-resistant lice were found in Tambov too but constituted 22.5%.

Body lice show resistance to permethrin and d-phenothrin in the dipping test. Sensitivities of different lice samples however vary greatly (Table 1 and 2). It was not possible to obtain the 95% death rate in the part of high RR populations. Toxicity increased linearly at higher doses but showed a plateau of 25-50% mortality at permethrin concentrations of 0.005-0.5%. This type of response is typical for mixed populations containing resistant individuals.

Permethrin resistance of lice is unrelated to their sensitivity to organophosphates (fenthion, malathion) and neonicotinoids (imidacloprid). Synergism with enzyme inhibitors in permethrin-resistant body lice indicates that in some cases enhanced metabolism is involved in pyrethroid resistance. Synergistic ratios were 3.3 to 52.0 for MO inhibitors, 5.2 to 7.4 for Est, and about 4 for G-S-T (Table 2). Slight synergism of permethrin combined with detoxifying enzyme inhibitors indicates that there is another resistance mechanism in addition to increasing enzyme activity (MO, Est and G-S-T). Since the use of synergists did not led to the full inhibition of resistance, lice probably posses knockdown resistance (*kdr*) as well. Lack of correlation between resistance and synergistic ratios supports this view. Cross-resistance to pyrethroids (permethrin, d-phenothrin) and organochlorine insecticide DDT also provides indirect evidence for *kdr*-resistance.

In Moscow the resistant haplotype was present in all body lice samples. The frequency of the pyrethroid resistance gene (T917I, L920F) was measured in 153 insects using RT-PCR. Of these, 101 (66.0%) were homozygous resistant, 18 (11.8%) homozygous susceptible, and 34 (22.2%) heterozygous. No colonies of exclusively permethrin-susceptible (SS) insects were found. Sequestering of the 1st and 3rd exons of the Vssc gene confirmed the specificity of the developed method of detecting T917I, L920F mutations in real time using PCR. The three mutations, M815I, T917I, and L920F, were detected only en bloc.

Table 1. Body lice susceptibility to insecticides.

Insecticides	RR at CL_{50} permethrin	n	Slope± SE	CL50 (95% CI) % a.i.	CL95 (95% CI) % a.i.
			Pyrethroids		
permethrin	17	360	1.80±0.19	0.017 (0.013-0.022)	0.09 (0.07-0.12)
	50	360	2.42±0.26	0.05 (0.038-0.065)	0.25 (0.19-0.33)
	80	360	1.73±0.17	0.08 (0.062-0.104)	1.0 (0.76-1.31)
	320	360	2.90±0.28	0.32 (0.25-0.42)	>1.0
	360	360	2.29±0.24	0.36 (0.28-0.47)	>1.0
d-phenothrin	90	360	2.19±0.76	3.0 (2.3-3.9)	>5.0
	320	360	-	>5.0	>5.0
			Organophosphates		
malathion	17	360	2.5±0.20	0.0045 (0.0035-0.0059)	0.022 (0.017-0.029)
	320	360	3.03±0.26	0.0050 (0.0038-0.0065)	0.025 (0.019-0.033)
fenthion	17	360	3.38±0.32	0.00012 (0.00009-0.00016)	0.00037 (0.00028-0.00048)
	320	360	3.47±0.73	0.00015 (0.00012-0.00020)	0.00040 (0.00031-0.00052)
			Neonicotinoids		
imidacloprid	25	360	2.71±0.55	0.0035 (0.0029-0.0041)	0.015 (0.012-0.020)
	360	360	2.59±0.27	0.0046 (0.0040-0.0052)	0.024 (0.018-0.031)

The data obtained indicate that the mechanism of lice permethrin resistance involves complex adaptation to pyrethroids. It includes at least two factors: *kdr* mechanism and non-specific resistance through the increase in the activity of detoxifying enzymes. Different lice populations may have the same overall resistance ratio, but the ability of their enzyme systems to increase their activity may differ.

Table 2. Effect of synergists on potency of permethrin in various body lice populations

Colony	n	Insecticide + synergist	CL_{50} (95% CI) (% a.i.)	SR	RR
Susceptible strain	420	permethrin	0.001 (0.0007-0.0013)	-	0
M158	420	permethrin	0.017 (0.013-0.021)	-	17
	420	+ PBO	0.009 (0.007-0.012)	1.89	9
	420	+ DEM	0.0045 (0.0035-0.0059)	3.78	4.5
	420	+ DEF	0.002 (0.0015-0.0026)	8.50	2
M109	420	permethrin	0.045 (0.035-0.059)	-	45
	420	+ PBO	0.054 (0.042-0.070)	0.83	54
M214	420	permethrin	0.19 (0.14-0.25)	-	190
	420	+PBO	0.20 (0.15-0.26)	0.95	200
	420	+DEM	0.21 (0.16-0.28)	0.90	210

M147	420	permethrin	0.23 (0.18-0.30)	-	230
	420	+ DEM	0.10 (0.07-0.12)	2.30	100
	420	+ DEF	0.18 (0.14-0.23)	1.28	180
M206	420	permethrin	0.30 (0.23-0.39)	-	300
	420	+ PBO	0.09 (0.07-0.12)	3,33	90
	420	+ DEM	0.075 (0.058-0.098)	4.00	75
	420	+ DEF	0.058 (0.045-0.075)	5.17	58
M36	420	permethrin	0.36 (0.28-0.47)	-	360
	420	+ PBO	0.23 (0.18-0.30)	1.57	230
	420	+ DEM	0.13 (0.10-0.04)	2.77	130
	420	+ DEF	0.24 (0.18-0.31)	1.50	240
M74	420	permethrin	0.37 (0.28-0.48)	-	370
	420	+ PBO	0.09 (0.07-0.12)	4.11	90
	420	+ DEM	0.35 (0.27-0.46)	1.06	350
M168	420	permethrin	0.50 (0.38-0.65)	-	500
	420	+ PBO	0.40 (0.31-0.52)	1.25	400
	420	+ DEM	0.51 (0.40-0.67)	0.98	510
M169	420	permethrin	0.51 (0.40-0.68)	-	510
	420	+ PBO	0.31 (0.23-0.39)	1.65	310
M207	420	permethrin	0.52 (0.40-0.68)	-	520
	420	+ PBO	0.01 (0.008-0.013)	52.00	10
	420	+ DEM	0.12 (0.09-0.16)	4.33	120
	420	+ DEF	0.07 (0.05-0.09)	7.43	70

CONCLUSIONS

Because resistant lice were detected in all colonies, it is speculated that resistant lice are spread extensively in Russia, both in large cities and smaller towns. Toxicological and genetic data indicate that the principal mechanism of body lice resistance to permethrin is nervous system insensitivity as a result of mutations in the *Vssc1* gene. A weak enzyme-based permethrin resistance mechanism was identified. Our results indicate multiple mechanism of pyrethroid resistance in lice populations.

ACNOWLEDGMENTS

This work was in part financially supported by the Russian Foundation for Basic Research (grant № 13-04-01657).

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