Laboratory Studies of Susceptibility and Resistance to Insecticides in *Pediculus capitis* (Anoplura; Pediculidae)

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ABSTRACT The susceptibility of local head lice to permethrin, sumithrin, deltamethrin, and carbaryl was determined by laboratory bioassays in field-collected colonies. Head lice collected from the infested heads of children 6–12 yr old were tested within 3 h of collection. The longest survival of control insects in the laboratory was obtained by keeping them in the dark at 18°C and 70–80% RH. The base line susceptibility data obtained for insects collected from children not treated for lice, the reference colony, showed that deltamethrin caused the highest mortality of the insecticides tested (LC_{50}, 0.06%). Permethrin, sumithrin, and carbaryl showed no significant difference in mortality (superposition of confidence intervals), being 10 times lower than that caused by deltamethrin. All field-collected lice required a higher LC_{50} of permethrin than the reference colony. Resistance levels varied from 3 to >100 for colonies that were taken from children treated with anti-lice products. Lice colonies with permethrin resistance showed resistance to sumithrin and deltamethrin, but resistance was not observed to the carbamate carbaryl.

KEY WORDS *Pediculus capitis,* insecticide resistance, cross resistance, laboratory bioassay

INFESTATION WITH THE head louse *Pediculus capitis* (De Geer) has increased worldwide since the middle of the 1960s; the number of cases of head and body lice infestation has been estimated to be >100 million (Mumcuoglu et al. 1990).

Synergized pyrethrins, pyrethroids, carbaryl, malathion, and lindane are effective agents for the treatment of head lice. Resistance of head lice to organochlorine insecticides was reported by Maunder (1971) and resistance to synergized pyrethrins and malathion was reported for body and head lice by Cole and Clark (1961), Blommers and Van Lennep (1978), and Blommers (1979). Permethrin resistance in head lice was reported by Rupes et al. (1995) and by Mumcuoglu et al. (1995). Baseline susceptibility data for *P. capitis* has been hampered by short survival times when the lice are removed from the host and also the difficulty in collecting and rearing sufficient numbers to conduct laboratory tests.

In Argentina, permethrin is the major insecticide used in pediculicide formulations, and treatment failures have been reported by Mássimo et al. (1996). The current study was conducted to determine the susceptibility of local head lice populations to permethrin and deltamethrin and to determine if cross-resistance to other insecticides which had not yet been used for the control of head lice in Argentina occurred. Field-collected lice were used to establish baseline susceptibility levels in the city of Buenos Aires.

Materials and Methods

Lice. Lice were collected from heads of infested children 6–12 yr old. Lice were collected from elementary schools and children hospitals located in different areas of Buenos Aires. Head lice collected within a 2-h period were transported to our laboratory. Adult males and females and large nymphs were selected for bioassays because no differences in susceptibility were reported by Mumcuoglu et al. (1990). The lice were examined carefully with a stereoscopic microscope (NIKON SMZ 10), and damaged specimens (e.g., missing ≥1 leg) were discarded (WHO 1982). All lice used in the baseline susceptibility studies were tested within 3 h after collection and were protected from sunlight and heat.

Identification of Field Samples. The Argañaraz reference lice colony was initiated from collections from 8 infested children in the family that never received pediculicide treatment. These lice had the greatest susceptibility to insecticides.

In preliminary bioassays, we found that lice collected from the heads of children in the same classroom or children living in the same home had similar levels of insecticide susceptibility. Therefore, lice were grouped in colonies according to the school and according to the home in which they had been collected. Table 1 shows the data of the lice colony used. 3 Febrero and Lujan Porteño colonies were collected from children in schools located in high-level socioeconomic areas and had previous treatment with in-

Table 1. Sources of lice colonies in Argentina used for bioassays

Colony	Place	Source of head lice	Socioeconomic level	Previous treatments
Argañaraz	Cassanova, Buenos Aires	Home	Low	None
Vela-Baez	Morón, Buenos Aires	Home	Medium	Occasional
3 Febrero	3 de Febrero, Buenos Aires	School	High	Many
Isla Maciel	Isla Maciel, Capital Federal	Hospital	Medium	Occasional
Luján Porteño	Flores, Capital Federal	School	High	Many
Palermo	Palermo, Capital Federal	School	High	Occasional

secticides. Vela-Baez , Palermo, and Isla Maciel colonies were started from collection in medium-level socioeconomic areas and that had occasional treatment with insecticides.

Chemicals. Technical grade permethrin (42.5% *cis* and 54.2% *trans*) (Chemotecnica Sintyal, Buenos Aires, Argentina); deltamethrin (97% [AI]) (AgrEvo, Buenos Aires, Argentina); carbaryl (98% [AI]) (Rhone Poulenc, Lyon, France); sumithrin (94.4% [AI]) (Sumitomo Chemical, Osaka, Japan); dioctylphtalate (98%) (Aldrich, Milwaukee, WI) were used. Insecticide concentrations used for bioassay ranged from 0.1 to 50% of active ingredient in stock solution. All solvents were analytical grade (Merck, Buenos Aires, Argentina)

Survival of Untreated Lice. Head lice were exposed to different environmental conditions to determine those that prevented mortality in the untreated groups. Batches of 10 lice each were released onto a clean piece of Whatman No. 1 filter paper (7 cm diameter, Whatman, Hillsboro, OR) that had been placed at the bottom of a plastic petri dish that was used as an experimental unit. Dry filter paper was used to determine the effect of low humidity (40–50% RH), and filter papers sprayed with 0.1 ml of water were used to determine the effects high humidity (70–80% RH). Experimental units (petri dishes) were placed in an environmental chamber (Lab-Line, at 18.0 ± 0.5 or 25.0 ± 0.5°C) in the dark. Mortality was established as the inability of the lice to walk on the filter paper. In all determinations, at least 3 replications were used.

Bioassay. Insecticide activity was evaluated by confining the lice on insecticide-impregnated filter papers, according to the method described by Maunder (1971) and Blommers and van Lennep (1978) and recommended by the World Health Organization (WHO 1981).

Stock solutions of each insecticide in dioctylphthalate were prepared from the technical products. One mililiter of stock solution was diluted in 2 ml chloroform to obtain the desired concentration. Then 0.4 ml of each concentration of final solution was spread over a piece of Whatman No. 1 filter paper (7 cm diameter) and dried for 24 h.

Batches of at least 10 lice were confined during 1 h on the impregnated papers by glass rings (4 cm diameter). At least 4 exposure concentrations were used, 3 of which caused partial mortality. At the end of the exposure period, the treated insects were released onto a piece of clean Whatman No. 1 filter paper (7 cm diameter) placed at the botton of a plastic petri dish, and 0.1 ml water was placed on the filter paper to provide humidity for the insects. The experimental units were placed in a Lab-Line environmental chamber at 18.0 ± 0.5°C and 70–80% RH in the dark. Untreated insects were placed on Whatman No. 1 (7 cm diameter) filter paper that contained 0.4 ml of a dioctylphthalate chloroform solution (1:2). Dead and live lice were recorded after 18 h. In all experiments, at least 3 replicates of each concentration were used.

Statistical Analysis. Mortality data for each concentration were used to determine the LC_{50} (lethal concentration for 50% of exposed lice) using probit analysis (Litchfield and Wilcoxon 1949). The LC_{50} was expressed as the percentage of active ingredient contained in the stock solution. The resistance ratio was calculated as the ratio between LC_{50} of the lice collected from different areas and the LC_{50} of the reference laboratory colony.

Results

Survival of Untreated Insects. Mortality of Argarañaz strain lice at different environmental conditions was evaluated to optimize bioassay variables. The results of the laboratory exposure of untreated lice to 18.0 ± 0.5 and 25.0 ± 0.5°C and 40–50 and 70–80% RH showed that mortality recorded after 18 h varied between 2.5 ± 2.5% and 92.0 ± 6.8%. Untreated louse mortality at the low temperature was less than at the higher temperature, and mortality at the higher humidity was less than at the lower relative humidity. An average mortality of 2.5% was observed at 18.0 ± 0.5°C and 70–80% RH. Average mortality of 92.0% occurred at 25.0 ± 0.5°C and 40–50% RH.

Baseline Susceptibility. The concentration-mortality LC_{50} for the lice used as the susceptible reference population to permethrin, sumithrin, deltamethrin, and carbaryl is shown in Table 2. Based on the LC_{50}

Table 2. Susceptibility of reference colony exposed to 4 insecticides in laboratory bioassays

Insecticide	*n*	% LC_{50}	Slope ± CL	95% FL
Permethrin	60	0.55	4.11 ± 0.22	0.32–0.94
Sumithrin	50	0.63	2.13 ± 0.11	0.34–1.66
Deltamethrin	50	0.06	3.64 ± 0.21	0.04–0.12
Carbaryl	70	0.35	2.57 ± 0.24	0.31–0.42

Adults and large nymphs were exposed during 1 h to treated filter papers. Mortality was recorded after 18 h. Values are expressed as percentage of active ingredient in stock solution.

Table 3. Susceptibility values (LC_{50}) and resistance ratios to permethrin in head lice colonies

Colony	n	% LC_{50}	Slope ± CL	95% FL	Resistance ratio
Vela-Baez	50	12.75	5.17 ± 0.45	6.36–23.21	23.17
3 Febrero	70	>50	—	>50	>90.9
Isla Maciel	110	1.65	1.86 ± 0.08	0.21–4.60	3.0
Lujan Porteño	50	>50	—	>50	>90.9
Palermo	120	6.20	1.85 ± 0.15	3.20–10.81	11.3

Adults and large nymphs were exposed during 1 h to treated filter papers. Mortality was recorded after 18 h. Values are expressed as percentage of active principle in stock solution. LC_{50} reference strain, 0.55%.

response, deltamethrin caused the highest mortality at the lowest concentration followed by carbaryl. Permethrin and sumithrin showed no significant difference in mortality (superposition of confidence intervals).

Comparative Susceptibility. The results of comparative susceptibility tests for field-collected head lice against permethrin are shown in Table 3. The lice showed different resistance ratios ranging from 3.0 to >90.9 in field-collected samples. Exposure of resistant lice to filter papers impregnated with 50% permethrin in stock solution caused 0% mortality.

Results of comparative tests on lice colonies against sumithrin, deltamethrin, and carbaryl are given in Table 4. All permethrin-resistant colonies also showed resistance to deltamethrin, another pyrethroid used as a pediculicide in Argentina and resistance to sumithrin, an alternative pyrethroid never used before as a pediculicide. In the resistant 3 Febrero colony, the level of resistance for the alternative insecticide sumithrin was lower than the one for permethrin. In resistant Vela Baez, Isla Maciel, and Palermo colonies, sumithrin resistance was higher than the one found for permethrin. High resistance levels to permethrin in the Lujan Porteño colony (>100) also showed high cross-resistance to sumithrin (>100). Pyrethroid-resistant colonies showed low or no resistance to carbaryl, a carbamate insecticide.

Discussion

Even though insecticides fail to control head lice, in the field, few papers have been published concerning the susceptibility–resistance of the insect in the laboratory, mainly because of the great difficulty that exists in maintaining a permanent laboratory colony and the short life expectancy of this insect when removed from the host. Blommers and Van Lennep (1978) reported that field-collected lice could be used to provide a country-wide indication of susceptibility levels. They found that control mortality varied from 0 to ≥20% during incubation in the dark at 26 ± 1°C and 60–70% RH during 24 h. Our results showed that the longest survival of field-collected head lice in the laboratory was obtained by keeping them in the dark at 18°C and 70–80% RH for 18 h. The 2.5% average control mortality obtained under these conditions was comparable with that reported by Rupes et al. (1984), who found 2.9% mortality after 16 h of exposure to bunches of polyamide fibers in testing susceptibility of head lice to insecticides collected in Czechoslovakia. These results showed that environmental laboratory conditions had a strong influence on the average control mortality and was probably caused by the high relative humidity and lower temperature preventing dehydration when the lice were not on the host.

The susceptibility-base data determined in the reference colony showed that deltamethrin had the highest toxicity of the tested insecticides (LC_{50}, 0.06%). Permethrin, sumithrin, and carbaryl showed no significant differences in toxicity (almost 10 times lower than that for deltamethrin). These results are in accordance with those obtained by Mumcuoglu et al. (1990), who evaluated the susceptibility of head lice to malathion, deltamethrin, permethrin, fenitrothion, and dieldrin collected from children in Israel. They found that deltamethrin had the highest knock-down effect, followed by permethrin and malathion.

All of the field-collected samples from Buenos Aires showed resistance to permethrin and deltamethrin when compared with the reference colony. The resistance levels varied from 3.0 for the Isla Maciel colony to >90.9 for the Lujan Porteño and 3 Febrero colonies. Given the past history of pediculicide formulations used in Argentina (most of them contain permethrin and 1 contains deltamethrin as the insecticide) in infested children of Lujan Porteño and 3 Febrero schools, the results were not surprising. In fact, permethrin resistance in head lice was reported by Coz et al. (1993) in France and by Rupes et al. (1995) in Czech Republic and by Mumcuoglu et al.

Table 4. Resistant ratio (RR) to insecticides in permethrin-resistant head lice colonies

Colony	Deltamethrin, % LC_{50} (95% CL)	RR	Sumithrin, % LC_{50} (95% CL)	RR	Carbaryl, % LC_{50} (95% CL)	RR
Vela Baez	0.52 (0.39–0.70)	8.7	17.7 (15.6–20.1)	28.1	1.67 (1.52–1.84)	4.8
3 Febrero	1.54 (1.09–2.20)	25.7	28.3 (20.5–54.9)	44.9	3.24 (2.75–3.80)	9.3
Isla Maciel	0.64 (0.56–0.73)	10.7	4.74 (4.22–5.33)	7.5	0.68 (0.60–0.78)	1.9
Luján Porteño	7.94 (6.21–11.45)	>100.0	>50.0	>100.0	0.17 (0.15–0.18)	0.5
Palermo	0.19 (0.15–0.23)	3.2	15.6 (14.1–17.3)	17.6	0.73 (0.63–0.85)	2.1

(1995) in Israel. In all cases, pyrethroid resistance has developed rapidly among head lice since permethrin was introduced as a pediculicide. Permethrin-resistant lice also showed sumithrin and deltamethrin resistance but did not show resistance to carbaryl. Cross-resistance between pyrethroid insecticides has been reported for several insect pests (Oppenoorth 1985, Picollo et al. 1992, Scott and Wheelock 1992), and little resistance to synergized allethrin was reported in a pyrethrin-resistant strain of *P. humanus* developed in the laboratory (Cole and Clark 1961). These authors reported that pyrethrin-resistant body lice showed little or no resistance to lindane or malathion. Resistance of head lice to organochloride insecticides (Maunder 1971) did not show cross-resistance to propoxur, malathion, or carbaryl. Resistance of head lice to permethrin in the Czech Republic was accompanied by cross-resistance to d-phenothrin and bioalethrin but kept susceptibility to malathion and pirimiphos methyl (Rupes et al. 1995). In all cases, the cross-resistance pattern between structurally related insecticides was caused by the fact that they had the same target and similar detoxification pathways (Zerba et al. 1987). Enhanced oxidative metabolism has been implicated as a major mechanism of resistance for all insecticide classes except the chlorinated cyclodienes (Scott 1990).

In the Vela Baez and 3 Febrero colonies, cross-resistant patterns between pyrethroid and carbamate insecticides could be caused by an increased activity of detoxification enzymes (e.g., mixed-function oxidases) that played an important role in the resistance mechanism. Otherwise, the Lujan Porteño colony showed a high resistance level to all of the pyrethroid insecticides evaluated but showed susceptibility to the carbamate, carbaryl, suggesting a more specific mechanism than the one described.

The pyrethroid resistance detected in head lice from 5 localities in the city of Buenos Aires can be considered to contribute to chemical control failure. To develop a strategy for the management of resistant head lice, studies concerning resistance mechanisms and possible tactics for its reversion are ongoing.

The results of these studies on the susceptibility and resistance of head lice to selected insecticides, including cross-resistance patterns, indicate the necessity to use alternative insecticides.

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