ORIGINAL ARTICLE

Carbamate Insecticides Resistance Monitoring of Adult Male German Cockroaches, *Blattella germanica* (L.), in Southern Iran

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Abstract

Background: The German cockroach, *Blattella germanica* L. (Dictyoptera: Blattellidae), has become resistant to many insecticides due to control failures in hospital and student dormitory settings of southern Iran.

Objective: This study was thus designed to detect and monitor carbamate resistance in two strains of German cockroach using lethal dose bioassay methods.

Methods: Wild dormitory (D) and hospital (H) strains were collected. Adult males were subjected to the jar exposure procedure. A range of concentrations based on the world health organization (WHO) standard concentration of carbamate insecticides (carbaryl, bendiocarb, propoxur) were used. For each insecticide, four to seven different concentrations leading to >0% and <100% mortality were assayed. Ten insects were placed in each jar and the exposure time of contact was held constant for 30 minutes. Mortality data (as LD_{50}) were assessed using probit analysis.

Results: Although both strains showed lethality values above those of the WHO standards, the H strain was more resistant to all of the three carbamate insecticides than the D strain, possibly due to the frequent and excessive dosage of these chemicals used in the hospital environment. The order of resistance for H strain was carbaryl > propoxur > bendiocarb. The ratio of LD_{50} in H strain to that of D strain for bendiocarb was about twice that of the other two insecticides indicating that German cockroaches were most susceptible to bendiocarb under both environments.

Conclusion: It is concluded that excessive reliance on carbaryl in both D and H settings has led to resistance.

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Keywords: *Blattella germanica*; Carbamate; Insecticide; Cockroach; Resistance

Introduction

The German cockroach, *Blattella germanica* L. (Dictyoptera: Blattellidae), is by far the most important indoor domestic pest in Iran. It is a major synanthropic insect in houses, hospitals and residential areas.[1, 2] It has been recognized as a pressing public health problem due to its potential to carry, disseminate and transmit human disease-causing pathogens as well as induction of asthma.[3] It is a small, highly proliferative, flightless and pestiferous vector which necessitates its control.[4]

Insecticide resistance poses an increasing problem to current pest control efforts.[5] By

definition, the resistant insect is one that "survives a dose of insecticide that would normally kill it".[6] This can be readily monitored with direct insecticide bioassays. Furthermore, widespread and heavy infestations of cockroaches might be treated with different chemical control formulations (residual spray, aerosol, dust, bait and gel) followed by integrated pest management to reduce their population overshoots.[7]

Multiple treatments and excessive doses of many of the currently-used insecticides under different settings particularly hospitals in recent decades have failed to curtail German cockroach breeding, probably due to development of resistance to many insecticide classes including the carbamate insecticides.[8]

Different levels of resistance to the carbamate insecticides in various strains of *B. germanica* have previously been well-documented in the United States,[9-11] Britain,[12] Cuba,[13] India,[14, 15] Malaysia,[16] Singapore,[17] China,[18] Taiwan,[3] South Korea,[19] and Iran.[20] Extensive insecticide resistance, high costs of labor and logistics, human health and ecological safety have been the major obstacles to a cost-effective integrated cockroach abatement program in Iran. It is thus essential to establish an efficient cockroach resistance abatement strategy using up-to-date information on the scale and nature of resistance.

Resistance monitoring is a crucial part of the resistance abatement approach by using valuable data on the responses of cockroach populations to insecticides. Following repeated use of organophosphorus and carbamate insecticides and persistent public complaints in recent years on the presence of indoor domestic pests with inherent potential to disseminate multidrug-resistant pathogenic agents, which is a significant concern for many health care professionals, this study was designed to detect and monitor carbamate resistance in field-collected strains of German cockroach by using lethal dose bioassay methods. To the best of our knowledge based on the most recent review of literature, this is the first report to monitor carbamate insecticides resistance in field strains of adult male German cockroach from the southern Iranian city of Shiraz.

Materials and Methods

Cockroach Strains

Two wild German cockroach strains were used in this study: a students' dormitory strain (D) which was considered to be susceptible to insecticides and a resistant strain (H) collected from a public training hospital (SFH) in Shiraz (52°30'E, 29°39'N, at an altitude of 1491 m above the sea level), the capital city of Fars province, south of Iran. This is a major tourist attraction city with numerous ancient monuments as well as modern and inexpensive health care facilities and centers, which are often crowded with people from other provinces and countries particularly those on the southern areas of the Persian Gulf. Any diminution in health index of any hospital would thus have long term expensive repercussions.

This hospital was treated with organophosphate, carbamate and pyrethroid insecticides in the past. Cockroaches were collected by trapping and hand-catch methods. Some 2500 adult cockroaches were used to foster the wild strains. All cockroaches were maintained in an insectarium at a constant temperature of 27±2 °C, a relative humidity of 60±10%, and a photoperiod of 12:12 (L: D) h. Each strain was kept in separate labeled glass rearing jars of the same size (240 ml), coated on the upper inner rim with petroleum jelly to prevent cockroaches from escaping and restricting them to the inner wall of each testing jar. Cockroaches were provided with bread, starch, soya, sugar cubes and water *ad libitum*. Tests were conducted only on the first generation adult males, since uniformity in gender would delete sex-biased differences in response to chemicals compared with those of females.

Insecticide Selections

Technical grade of chemical compounds, bendiocarb (Ficam®) 80% wettable powder (WP), carbaryl (Sevin®) 85% WP, and propoxur (Baygon®) 25% WP were used in this study. Stock solutions of these chemicals were prepared by dissolving the insecticides in analytical grade acetone as a solvent. Carbon dioxide gas was also used as an anesthetic. Baseline information on the susceptibility of *B. germanica* from the two study areas against the tested insecticides was not present as no similar study has previously been performed in this region.

Toxicity Bioassays

In order to assess resistance levels to the above-named carbamate insecticides, the field-collected cockroaches were subjected to the jar exposure procedure. The jar tests were conducted on the tarsal contact with insecticide residues which is the universally-accepted standard method for detecting resistance in the adult males of *B. germanica*.[21] This procedure involved continuous exposure of cockroaches within isometric glass jars (240 ml) coated with a known quantity of insecticide. For each insecticide, four to seven different concentrations leading to >0% and <100% mortality were used. The glass

scintillation jars were treated by adding 2 ml of the appropriate acetone insecticide solution to each one. They were then rotated evenly on a rotary mixer in a fume hood for one hour until the acetone evaporated. Ten cockroaches were then placed in each jar and the exposure time of contact was held constant for 30 minutes. Four replicates and one control (only acetone) were run for each population strain treated with or without the relevant insecticide. Cockroaches were then transferred to holding cups provided with ample food and water. Their mortality rates were scored within the next 24 h following the termination of exposure time. Mortality was assessed as the inability of the cockroaches to respond to probing with a paint brush. No control mortality occurred and mean percentage mortality and standard error were directly determined.

Statistical Analysis

Mortality data in the form of LD_{50} values and slopes from the replicates were assessed using probit analysis in SPSS.[22] The differences between LD_{50} values for each colony and its treatments were considered statistically significant only when 95% confidence limits of the LD_{50} values did not overlap. The proposal for this study was initially approved as to its design and statistical method by Shiraz University of Medical Sciences (SUMS), School of Health and Nutrition, Research Centre for Health Sciences, Scientific Board of Reviewers.

Results

In general, the hospital (H) strain of German cockroach, *B. germanica*, was more resistant than the dormitory (D) strain to all of the three tested carbamate insecticides (bendiocarb, carbaryl, propoxur). The latter strain was thus more susceptible or less resistant to the tested carbamate insecticides in this study. Although both strains showed lethality values above those of the WHO standards, the 'H' strain was less susceptible to all of the three carbamate insecticides than the 'D' strain, possibly due to the repetitive and excessive dosages of these chemicals used in the hospital environment. Different levels of resistance to these chemicals were noted in both dormitory- and hospital-collected strains of adult male German cockroaches with the resistance ranging from 0.7 to 62.4 mg/m^2 at LD_{50} levels. Based on the LD_{50} values, the order of resistance for both 'D' and 'H' strains was carbaryl > propoxur > bendiocarb. Mortality data as reflected by LD_{50} values and slopes showed that carbaryl had the greatest lethality value among all of the three carbamate insecticides in both settings (Table 1, Figure 1a). The lowest lethality value in dormitory and hospital strains belonged to bendiocarb (Figure 1b). Toxicity occurred with bendiocarb at a much lower dose than with propoxur but the minimum to maximum effect was achieved with propoxur over a very much narrower dose range. The order of toxicity levels in both strains for the three carbamate insecticides was thus as follows: bendiocarb > propoxur > carbaryl. The regression lines for propoxur indicated that at high doses the mortality of the two different strains was very close (Figure 1c). The treatment of carbaryl on 'H' strain of adult German cockroach produced the steepest (or sharpest = 5.82±2.19) slope among all of the three carbamate insecticides.

The ratio of LD_{50} in 'H' strain to that of 'D' strain for bendiocarb was calculated to be about 5.7 which was the largest value among all of the three carbamate insecticides (Table 2). This indicated low to moderate levels of resistance to bendiocarb. This was about twice that of the other two tested insecticides. The relevant ratios for propoxur and carbaryl between the two strains were similar (2.56).

Discussions

Arthropods transmit various pathogenic agents, which have increasing public health importance in Iran.[23] The threat of insecticide resistance represents the most formidable challenge to vector control measures.[5] The susceptibility of both strains ('H' and 'D') of adult German cockroach to the three tested carbamate insecticides in this study was evaluated by comparing the LD_{50} values estimated from tarsal contact jar method. It has previously been shown that the LD_{50} test sensitivity due to tarsal exposure of the adult German cockroaches to various insecticides could be more realistic for toxicological tests, since they depend on tarsal contact with insecticide residues and the amount of applied toxin could be precisely measured.[24, 25] This method was thus used in the current study. There was no mortality in the acetone-treated controls.

Although adult male German cockroach was used in this study, it appears that late-stage nymphs of *B. germanica* are significantly more tolerant to carbamate insecticides such as bendiocarb and propoxur.[18] Bioassays for resistance responses in the laboratory may not necessarily reflect control failure in the field due to discrepancy in the scale and nature of insecticide exposure under different settings.[18]

The possibility of prior insecticide exposure effects on 'H' strain of *B. germanica* leading to a synergistic outcome cannot be ruled out. Insecticide selection pressure could have been experienced in the field at some earlier time, resulting in a greater insecticide tolerance

Table 1: Lethal dose values for carbaryl, bendiocarb, and propoxur on dormitory 'D' and hospital 'H' strains of German cockroach

Insecticides	Concentration mg/m²	Corrected* test mortality %	LD_{50}(mg/m²)** Fiducial limits LCL UCL #	Slope±SE	Homogeneous X^2 (df) Probability	Regression equation
Propoxur (Baygon ®) D	3.1400	45.00	4.00846 2.38904 5.37134	1.8558±0.3806	1(2) 0.23562	Y=1.8558X +3.8810
	6.2800	62.00				
	12.5600	75.00				
	18.8400	95.00				
Bendiocarb (Ficam ®) D	0.2820	20.00	0.68110 0.50335 0.89044	1.8235±0.3305	1(2) 0.53662	Y=1.8232X +5.3041
	0.5660	50.00				
	1.1310	67.00				
	2.2610	80.00				
Carbaryl (Sevin ®) D	10.3620	5.000	24.2870 21.2377 27.5694	4.1462±0.5972	1(2) 0.82337	Y=4.1462X +0.7441
	20.7240	42.50				
	31.8600	67.50				
	41.4880	82.50				
Propoxur (Baygon ®) H	3.1400	2.500	10.26361 8.5487 12.5314	2.7996±0.4226	1(2) 0.06618	Y=2.7996X +2.1688
	6.2800	40.00				
	12.5600	52.50				
	18.8400	77.50				
Bendiocarb (Ficam ®) H	2.2610	15.00	3.90046 3.46706 4.51920	3.5824±0.6780	1(3) 0.6780	Y=3.5824X +2.8824
	2.7270	35.00				
	3.3920	42.50				
	4.5220	57.50				
	5.5220	70.00				
Carbaryl (Sevin ®) H	20.7240	5.000	62.37677	5.8171±2.1883	9(3) 0.00001	Y=5.8171X +(-5.4419)
	31.8600	7.000				
	41.4880	10.00				
	51.7780	15.00				
	61.4480	22.50				
	71.4480	82.50				
	81.4480	90.00				

* Abbott's formula; **Probit analysis; LCL: Lower concentration limit; UCL: Upper concentration limit; #at 95% confidence limit.

(and higher LD_{50}) compared with the wild (non-selected) population.

The fact that carbaryl had the greatest lethality (LD_{50}) value among all of the three carbamate insecticides in both settings indicated that *B. germanica* was relatively resistant to carbaryl insecticide under laboratory conditions. This resistance was very salient when a 10% increase in the concentration of carbaryl (from 61 to 71 mg/m²) led to an approximately fourfold increment in percentage mortality of adult German cockroach. Increased hydrolase activity appears to have a major role in carbaryl resistance of this insect.[26] In addition; the slope of the regression line reflects the type of response. That is, a steep dose-response regression line (almost like that of propoxur) indicates that the value of the slope will be large, and the response reflects a strong single effect such as binding to an enzyme with avidity.[27] Indeed, the carbamate insecticide, propoxur, was more effective in reducing the cockroach density by the first week compared with other insecticides.[15] This could be due to multiple factors. Firstly, propoxur LD_{50} is significantly associated with glutathione-S-transferase activity.[11] Secondly, cytochrome P450 monooxygenase is involved in the detoxification of propoxur.[18] Thirdly, both oxidative and hydrolytic enzymes are correlated with the detoxification of propoxur.[17] Finally, reduced cuticular penetrations are accompanied by increased levels of metabolism.[28]

The insecticidal activity of bendiocarb was the least as compared to any of the three tested carbamate insecticides. Low to moderate levels of bendiocarb resistance were reported in the hospital strains of western Iranian town of Kermanshah.[20] In this study, the hospital strains in the city of Shiraz were also found to exhibit low to moderate levels of bendiocarb resistance. In order to evade this resistance in the clinical settings, it

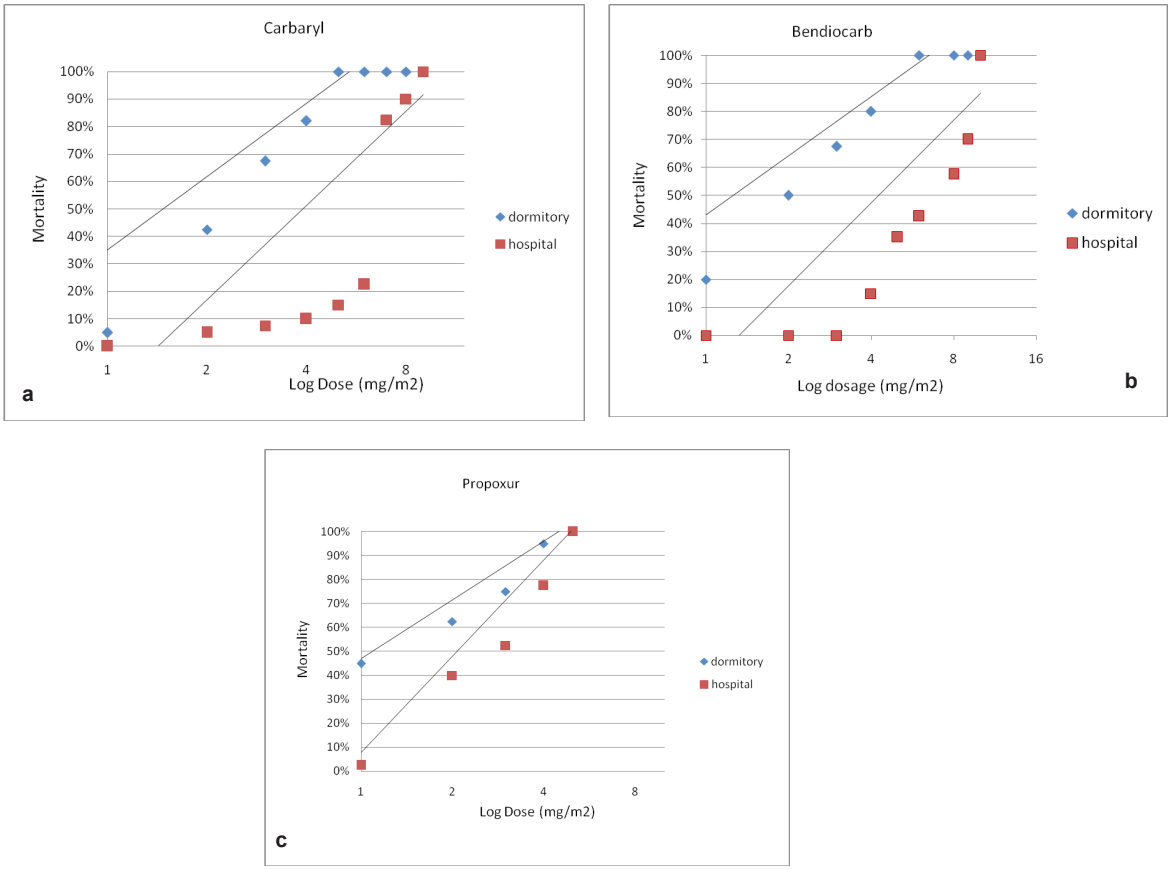

Figure 1: Probit regression lines due to lethal dose bioassays of carbamate insecticides such as carbaryl (a), bendiocarb (b), and propoxur (c) on German cockroach, *Blattella germanica*, adult males.

Table 2: Comparative lethal doses (LD_{50}) or concentrations and ratios of different carbamate insecticides on hospital (H) and dormitory (D) strains of German cockroach

Insecticide	Stand. Conc. (mg/m^2)	Effective Conc. (H) (mg/m^2)	Ratio of EC/SC (H)	Effective Conc. (D) (mg/m^2)	Ratio of EC/SC (D)	LD_{50} (D) (mg/m^2)	LD_{50} (H) (mg/m^2)	LD_{50} Ratio (H)/(D)
Bendiocarb	2.26	7.52	3.33	3.39	1.50	0.68	3.90	5.73
Carbaryl	10.36	100.44	9.69	51.45	4.97	24.29	62.38	2.57
Propoxur	6.28	25.12	4.00	18.84	3.00	4.00	10.26	2.56

is possible to switch on to pyrethroid insecticides coupled with piperonyl butoxide as a synergist.[29, 30]

Since the large physiological changes that often accompany resistance can influence other attributes of the insect vector (e.g. its viability), resistance management using basic rotations and mixtures of different insecticides should be considered.[6] The susceptibility of these strains to carbamate insecticides with respect to the standard WHO diagnostic dose reflects that they may be used in a rotation of different insecticides.[31] It is thus concluded that excessive reliance on carbaryl insecticide under both 'D' and 'H' settings has led to resistance.

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Conflict of Interest: None declared

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