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INSECT-BORNE DISEASES are a problem worldwide, made worse by increasing resistance of the vectors to insecticides. To control chagas disease in Bolivia, malaria in Africa or sandflies in the Middle East, which transmit leishmaniasis, it is essential that resistance management strategies are implemented.

A challenge for

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Insecticide resistance in public health pests

effective vector control

How resistance emerges, what mechanisms are involved, what is done to monitor resistance and what are possible options **to manage insecticide resistance**. These are essential aspects in dealing with disease-transmitting insect pests in Public Health.

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In public health many diseases are transmitted by arthropod vectors, e.g. mosquitoes (malaria, dengue fever, yellow fever, encephalitis, filariasis, West Nile fever and chikungunya), ticks (e.g. Lyme disease) and sandflies (leishmaniasis). It has been demonstrated in the past that the use of insecticides can dramatically reduce the risk of insect-borne diseases. This is well documented by the WHO and in numerous scientific investigations and reports, particularly concerning the most widespread and important disease, malaria.

Serious threat of insecticide resistance

Since the introduction of synthetic insecticides to control arthropod pests the selection pressure on populations has increased drastically, with many species developing mechanisms to withstand insecticide treatments. There is little doubt that

***Insecticide resistance** is defined as a heritable change in the sensitivity of a pest population that is reflected in the repeated failure of a product to achieve the expected level of control when used according to the label recommendation for that disease vector species.*

insecticide resistance has evolved to all classes of insecticides and is counteracting the control of many invertebrate pests of agricultural importance, disease vectors and other insects important in public health. The list of public health pests resistant to insecticides has been growing for decades and includes disease vectors such as many known mosquito species, fleas and ticks, as well as cockroaches, bedbugs, houseflies and more recently also Chagas bugs and sandflies.

Insecticide resistance is viewed as an extremely serious threat in crop protection and vector control, and is considered by many parties (industry, WHO, regulatory bodies and the public sector) as an issue that needs a proactive approach. In this direction the Insecticide Resistance Action Committee (IRAC) coordinates a private sector response to either preventing or at least delaying the development of insecticide resistance. IRAC not only facilitates communication and education on all aspects of insecticide resistance, but also promotes the development of resistance management guidelines *(see page 29: IRAC)*.

How resistant populations develop

Frequent applications of the same insecticide will select for those individuals in a population that are able to survive recommended levels of the compounds due to a stable genetic change. If one does not switch between different modes of action (or chemical classes) in application regimes, the number of less susceptible individuals will increase. Over a certain period of time (it may take months or even years) such one-sided insecticide administration will result in a resistant population. This is even more likely if combined with high reproductive potentials and short life cycles producing several generations per season.

Complicated by cross-resistance

In most cases a resistant pest population usually also shows resistance to other compounds within the same chemistry class, e.g. resistance against one pyrethroid usually results in resistance against the whole group of pyrethroids. This is known as cross-resistance. Sometimes, depending on the nature of the resistance mechanism, cross-resistance may even arise between chemical classes, e.g. organophosphates and carbamates (*see Fig. 2 page 14: Major biochemical mechanisms of resistance*).

Furthermore, resistance development due to selection pressure in disease vectors is complicated by an additional (sometimes neglected) aspect: frequent application of similar synthetic insecticides to control important agricultural pests may indirectly affect the susceptibility of insects important in public health.

Surveillance is essential

Once insecticide resistance is established in a population it can profoundly affect public health by the possible reemergence of vector-borne diseases. When resistance is monitored in suspected cases it is often too late. Focusing on surveillance wherever possible is essential in order to react proactively as soon as a regional population seems to be changing its susceptibility towards synthetic insecticides. For this purpose the WHO has published numerous monographs on methods to ensure the surveillance of resistance development to many different insecticides, e.g. by diagnostic dose bioassays for mosquitoes *(see list of monographs on www.who.int)*. A diagnostic dose tested in insecticide-coated glass vials usually provides 100% mortality of a susceptible population within one hour. In contrast, resistant populations survive such doses, at least to a certain extent.

Insecticide classes are limited

Another major aspect in terms of selection pressure on important public health insect species is the fact that only a limited number of insecticide classes are available. Only four different chemical classes of synthetic insecticides are (or have been) used to treat adult mosquitoes, i.e. organochlorines, organophosphates, carbamates and pyrethroids. Even the original compound (permethrin) of the synthetic pyrethroids has been available for more than 30 years (*see Fig. 1 below*).

It is important to note that these four chemical classes address only three different modes of action. Therefore, there is much less target-site diversity for the control of public health pests *(see diagram on cover flap)* than in the agricultural sector. In principle, all insecticidal classes have their biochemical target sites in the insect's

Insectides for mosquito control

Years	WHO approved insecticides		
1940-45	DDT		
1946-50	Lindane		
1951-55	Malathion		
1956-60			
1961-65	Fenitrothion	Propoxur	
1966-70	Chlorpyrifos-methyl		
1971-75	Pirimiphos-methyl	Bendiocarb	Permethrin
1976-80	Cypermethrin		
1981-85	Alpha-cypermethrin Lambda-cyhalothrin	Cyfluthrin Deltamethrin	 Bifenthrin
1986-90	Etofenprox		
1991-95			
1996-00			
2001-05			

Organochlorines · Carbamates · Organophosphates · Pyrethroids

Only a limited number of insecticide classes are available for adult mosquito control. No new malaria mosquito adulticide has been approved by the WHO in the last 15 years.

Fig. 1

central nervous system, i.e. cholinergic nerve transmission, which is one of the reasons why they act quite rapidly.

Pyrethroids have excellent properties

Insecticide classes used to control adult mosquitoes as malaria vectors have to meet specific requirements, i.e. excellent contact action, a rapid knock-down effect and selective toxicology. This is why the pyrethroids in particular were most successfully used in mosquito control over the last decades, with economic values of ca. 60% and 80% in residual use and space spray, respectively. Due to their excellent properties they account for 100% of global bednet treatments. Adding up all these numbers one could easily imagine that the pyrethroid selection pressure on many pests of importance in public health is considerable, particularly on malaria vectors. The risk of resistance development to pyrethroid insecticides in mosquitoes is therefore quite high.

Behavioral resistance

Insecticide resistance in mosquitoes is not always based on biochemical mechanisms such as metabolic detoxification or target-site mutations, but may also be conferred by behavioral changes in response to prolonged spraying programs. Behavioral resistance does not have the same importance as physiological resistance, but can be considered a contributing factor, leading to the avoidance of lethal doses of an insecticide. A behavioral response is either dependent or independent on a stimulus. If mosquitoes avoid a treated place due to sensing the insecticide it is considered to be a behavioral change dependent on a stimulus, whereas the selective occupation of an untreated area can be considered a stimulus independent response.

Mechanisms of resistance

Extensively studied in the past, resistance mechanisms in mosquitoes can be divided into two groups, metabolic (degradation of the active ingredient by detoxification enzymes) and target-site resistance (mutations in the target proteins). Both mechanisms can be quite specific, although there is a tendency for metabolic mechanisms to be more versatile with regard to cross resistance between chemical classes. A compilation of the major mechanisms of resistance and their respective importance for the different chemical classes used in malaria vector control is given in *Figure 2 (see page 14: Major biochemical mechanisms of resistance)*.

Resistance to pyrethroids and organochlorines

Mosquitoes' resistance to pyrethroids and DDT is either conferred by a mutation in the voltage-gated sodium channel (kdr) or by elevated levels of microsomal monooxygenases. Several monooxygenase genes have been associated with pyrethroid resistance, particularly relating to permethrin. DDT resistance is also specifically conferred by the so-called DDT-dehydrochlorinase, a glutathione S-transferase. In addition, rdl (resistance to dieldrin, a mutation in the GABA-gated chloride channel) results in resistance to organochlorines other than DDT. In contrast to pest insects of agricultural importance, esterases have not yet been shown to play a major role in conferring pyrethroid resistance in mosquitoes.

Resistance to organophosphates and carbamates

In contrast to pyrethroids, over-expression of esterases by gene amplification provides considerable organophosphate (and to a certain extent carbamate) resistance in mosquitoes. This has been reported as an evolutionary response to selection by organophosphates and carbamates. A second mechanism of importance is MACE. Both organophosphates and carbamates are affected by this target-site mutation in acetylcholinesterase. Monooxygenases only play a minor role in organophosphate and – if any – in carbamate resistance.

Monooxygenase-based cross resistance to carbamates has been described as unusual in mosquitoes. It was only reported for propoxur as a notable exception, whereas bendiocarb against the very same mosquito strains gives excellent control at diagnostic doses. This is likely to be due to the structural uniqueness of bendiocarb, which carries a bis-methylated methylenedioxy-motif possibly resilient to attack by insect cytochrome P-450 enzymes. In other words, although these carbamates share the same mode of action as organophosphates they are structurally different and may select for different mechanisms of resistance.

Resistance management a challenge

The management of insecticide resistance, or more precisely, the management of arthropod pest susceptibility is crucial. It should be considered as one of the most challenging issues in modern applied entomology. The effective management of malaria vectors by only a limited number of insecticide chemical classes is a challenge in itself. As briefly outlined here, the chemical options for a resistance management strategy in adult mosquitoes are limited. Currently there are only four chemical classes of insecticides addressing just two different insect target sites!

Larvicides provide new options

Mosquito larvae carry the same resistance genes as adults. Therefore, they are also resistant to the same compounds, although the extent of the resistance might differ between adults and larvae. However, there are also various new classes of larvicides for controlling immature insect populations *(see page 45: Chikungunya)*. One class, called insect growth regulators (IGRs) has various biological modes of action. So far IGRs are usually detoxified by metabolic enzymes such as

Photo: gettyimages

ENCEPHALITIS transmitting mosquitoes often breed in rice fields – use of agricultural insecticides can trigger the development of resistance in vector control.

microsomal monooxygenases. No cases of target-site resistance have yet been described for any IGR. Biologicals such as bacterial endotoxins are also an option. Resistance to *Bacillus sphaericus* toxin has been described in field populations and laboratory selected strains of different species of mosquitoes. This resistance is due to the toxin failing to bind to the specific mid-gut receptors, which is caused by several mutations in the binding protein. Usually, no cross-resistance to *Bacillus thuringiensis* (BTI) is seen, because the toxins of these bacteria bind to different mid-gut receptor proteins.

Rotation recommended

Most recommendable is the rotational use of chemicals with different modes of action, rather than alternating members of one chemical class or different chemical classes addressing the same target site. Possible implications of resistance mechanisms on such strategies can be worked out from Figure 2. For example, the presence of kdr resistance renders DDT and pyrethroids less effective, whereas carbamates such as bendiocarb or organophosphates such as fenthion can still be used. If the MACE mechanism is not a problem, one may even think about the rotational use of carbamates and organophosphates. This might increase the chances of regaining pyrethroid susceptibility. However, this should be carefully monitored.

Furthermore, it seems much more likely to select for cross-resistance to organophosphates than to pyrethroids when using carbamates in alternation

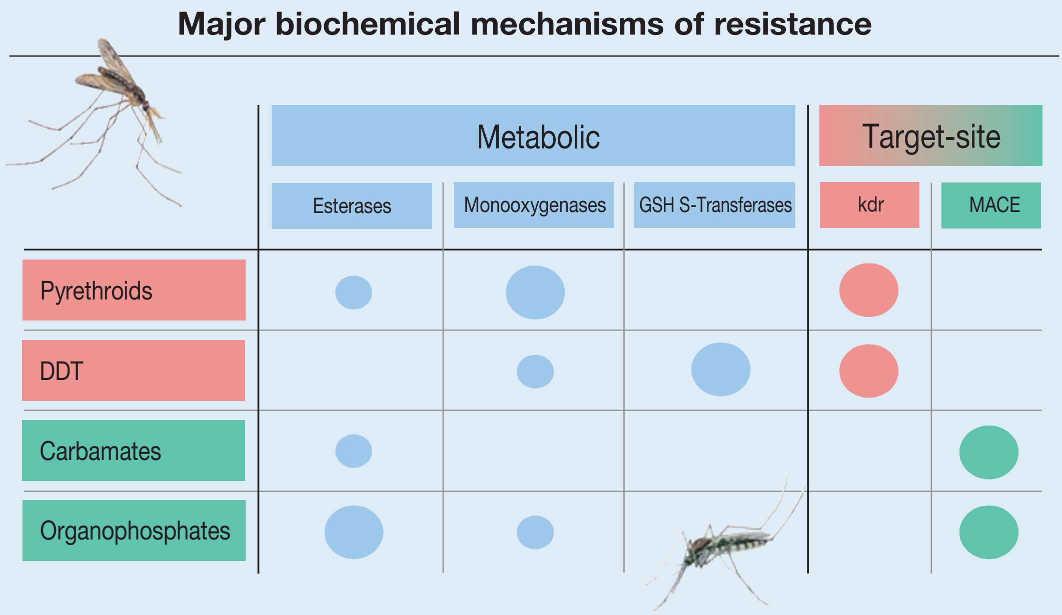

Fig. 2: The metabolic detoxification enzymes described to confer insecticide resistance in mosquitoes are: esterases, monooxygenases and glutathione S-transferases (in blue). Two major target-site mechanisms are relevant today: kdr (knock-down resistance), a mutation in the voltage-gated sodium channel (in pink), and MACE (modified acetylcholinesterase) (in green). The respective importance of each resistance mechanism is indicated by the size of the circle.

with pyrethroids, particularly due to possible selection for MACE-based resistance. In conclusion, both organophosphates and carbamates, particularly bendiocarb are useful as rotational partners in resistance management strategies in order to sustain pyrethroid susceptibility in mosquitoes.

Article on the enclosed Public Health CD-ROM

CONCLUSION

Effective long-term resistance management is necessary, but many factors need to be considered to successfully implement strategies. This is not only achieved by the availability of insecticides but is also driven by other factors, e.g. training courses and educational material on disease prevention, or by vector control personnel in general educating management principles, to ensure proper implementation and surveillance. Of course, new active ingredients with new modes of action would be most welcome in order to diversify the vector control toolbox and extend the life cycle of all available insecticides, thus lowering the risk of reemerging vector-borne diseases.

1st International Workshop on Resistance Management

South Africa, 29-30 June 2004

A meeting on malaria vector resistance was organized in South Africa by the Medical Research Council. The objectives of the meeting were to review the current situation of resistance in Southern Africa, mechanisms involved and practical implications for vector control in the region. This meeting was sponsored by Bayer Environmental Science.

About 40 people participated from 9 countries, including South Africa, Swaziland and Mozambique, and managers of 5 Malaria Control Programs, representatives of WHO (HQ & AFRO) and scientists involved in vector resistance (UK, Mexico). Detailed questions for further research were addressed.

Some recommendations made during the meeting:

- WHO to coordinate the development of guidelines for resistance management with special reference to Southern Africa, and with the target audience being program managers.
- Industry to develop new formulations (OPs, carbamates, pyrethroids), which would last for 12 months on all surfaces, including mud and cement.
- Pesticide industry to contribute to resistance monitoring within the framework of product stewardship (modalities to be further discussed).

The document regarding "Recommended actions to support research and control strategies" can be found on the enclosed Public Health CD-ROM