DePin et al., Vector Biol J 2018, 3:2
DOI: 10.4172/2473-4810.1000130

Vector Biology Journal

Research Article

A SCITECHNOL JOURNAL

Susceptibility of *Anopheles gambiae Sl* to the Insecticides in Praia, Cape Verde: A Country in the Pre-Elimination of Malaria

Adilson José DePina[1,2]*, Moussa Namountougou[3], Silvania Veiga Leal[4], Isaias Baptista Fernandes Varela[4], Davidson Daniel Sousa Monteiro[4], Celivianne Marisia Ramos de Sousa[4], Aderitow Augusto Lopes Gonçalves[4], António Lima Moreira[5] and Ousmane Faye[6]

Abstract

Background: Success for malaria control and elimination implies effective vector control strategies. In Cape Verde, country in the pre-elimination phase of malaria, the Indoor Residual Spraying with insecticides is the main strategy in the vector control. The objective of this study was to assess the level of insecticide sensitivity of *Anopheles gambiae sensu lato*, populations from Praia, Cabo Verde, to the principal insecticide recommended for IRS, the deltamethrine 0.05%, 0.25% and 0.5%, Bendiocarb 1% and malatahion 5%.

Methods: Larvae of *Anopheles gambiae* s.l were collected from different sites in Praia and reared to adult stages in the medical entomology lab. The sensibility/resistance status was assessed using the WHO bioassay test kits for adult mosquitoes. The result of studies was analysed, determining the mortality rate for different insecticide, the KD determined with Log-Probit and others data analysis with Excel.

Results: A total of 693 females of *An. gambiae* s.l. was used to determine the sensitivity status. The results of demonstrate an increase of KDR times more pronounced with the lowest concentration of deltamethrin (0.05%), compared to the highest concentrations (0.25% and 0.5%). We found a resistance to deltamethrin 0.05% and Malathion 5%, although less marked with mortality rates above 80%. However, the vector population were find completely susceptible to deltamethrin 0.25%, deltamethrin 0.5% and to bendiocarb 1%.

Conclusion: This study provides updated information about the sensibility of *An. gambiae* s.l. to insecticides in Praia, Cape Verde. The constant use of this insecticides, for long years, in vector control as well in agriculture, could be the cause to the selection pressure for resistance. We recommend the use of this findings in the definition of the vector control strategies and to the implementation of a routine resistance monitoring to the malaria vector control in the country.

Keywords

Anopheles gambiae s l; Resistance; Malaria elimination; Cape Verde

***Corresponding author:** Adilson José De Pina, Malaria Elimination Program; CCS-SIDA, Ministry of Health, CP: 855, Praia, Cabo Verde, Tel: +238 2603779; Fax: +238 2618576; E-mail: Adilson.Pina@ccssida.gov.cv

Received: July 25, 2018 **Accepted:** August 08, 2018 **Published:** August 28, 2018

Introduction

Although preventable and curable, malaria continues to cause millions of cases. In the last years, the number of cases of malaria and countries reporting cases was decreased. In total, 17 countries eliminated the disease between 2000-2015 (i.e. attained zero indigenous cases for 3 years or more) and two countries, Kyrgyzstan and Sri Lanka these were certified by WHO as malaria free in 2016 [1].

With these encouraging data, it is believed to be possible, eliminating malaria from 10 countries, by 2020 [2]. And, one of them is Cabo Verde, where the transmission of malaria is unstable, normally during the period September-December and sometimes marked by epidemic outbreaks. The majority of malaria cases in the country are reported in the main island, Santiago, which presents the highest risks due to the autochthones cases and the presence of the vector [3].

Anopheles gambiae s.l. was the first species of culicidae identified in Praia, Cabo Verde, in the year 1909. Years later, the same species was identified in Pedra Badejo, interior of Santiago and later on São Vicente island. As early as 1947, this specie was reported in several localities of Sal and Boavista islands and in 1959 was reported on the islands of Brava and Maio [4]. In Cape Verde, until now, the only vector specie associated with malaria parasite transmission is *Anopheles arabiensis*, the only member of *Anopheles gambiae* complex, that was identified by cytogenetic analysis in samples obtained from various islands [5] in various studies [6-10]. This species is characterized by an anthropophilic, endo-exophageal preference and marked exophilia in the country [6,7,10].

In context of study of the feasibility of malaria pre-elimination, several factors have been identified as able to promote the elimination of this disease in the Cape Verde Islands. These include the epidemiological situation, the subtropical dry climate, the simplicity of the system vector (a single vector present), strong political commitment and funding opportunities. The current vector control methods are focused primarily on larval control based on the use of larvivorous fish, temephos, intermittent drying of some water tanks and others environmental interventions. Aimed to eliminate malaria, these activities of anti-larval control had been, reinforced by intra-domiciliary spraying at national level, twice a year, during the rainy season. To the control of adult mosquitoes, IRS with deltamethrin WP 0,5% is the key strategy in use in the country, with two annual spraying campaigns in the rainy season [11].

During the last years (2010-2017), 528 malaria cases were registered in the city of Praia, the capital of Cabo Verde, representing 96% of cases at national level. This demonstrate the importance of the city regarding the incidence of malaria in the country and deserve special attention in the context of elimination of the disease in the country [3].

One of the crucial intervention to be considered in the malaria control and elimination process is the understanding of aspects related to the vectors's bio ecology, which includes the susceptibility to insecticides. Hence, the interest in carrying out this present study in the city of Praia, during the 2017 malaria outbreak, to support the Ministry of Health of Cape Verde and WHO in obtaining data about the susceptibility of *Anopheles gambiae* s.l. to insecticides. The data

generated will be helpful for implementation of effective and targeted strategies.

Materials and Methods

Study area

The current study was conducted during September and October 2017, in Praia, capital of Cabo Verde, Santiago Island. Praia is located in the southern part of Santiago (Figure 1), (Latitude 14.932 °, longitude -23.513° and 70 m of average altitude) where resides around 30% of the country's population [12]. Located in Santiago Island, Praia with the temperature generally ranging from 21°C to 27°C, rarely below 20°C or above 28°C. The warm season remains for 3.3 months, (August to November) with daily mean maximum temperature above 26°C.

The precipitation, in the capital varies throughout the year. The highest rainfall season lasts 2.4 months (between August to October), with daily precipitation probability above 13%. The dry season lasts 9.6 months, (between October to August) and the minimum probability of a day with precipitation is 0% on June. The rainy season lasts 6.1 months (between July-February), with at least 13 mm of 31-day continuous rainfall.

Praia has extreme seasonal variation about humidity. The hottest period of the year lasts 9.3 months, (March-January) in which the comfort level is muffled, oppressive or extremely humid at least 48% of the time. And the winds are generally moderate from the northeast reaching an average velocity of 3 m/s [13].

Mosquito collection and identification

In this present study mosquitoes were field collected as larvae or pupae. The samples were collected from different breeding sites of *An. gambiae* s.l., such as temporary stagnant water pool, edges of slowly flowing stream and irrigation sites in the city of Praia. The larvae were transported to the medical entomology laboratory in the National Institute of Public Health and reared to the adult stage in the insectarium conditioned with temperature at 28 ± 2°C and relative humidity at 80 ± 10%. The adults belonging to *Anopheles gambiae* complex, identified by morphological characteristics using the dichotomous keys [4] were selected to be used in the bioassay.

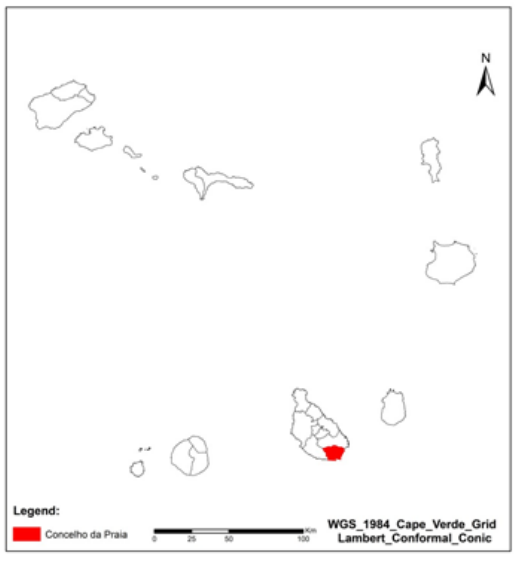

Figure 1: Localization of Praia, capital of Cabo Verde. Adapted by the authors.

Resistance bioassay

Adult susceptibility assays were carried out with three insecticides representative of three classes available for use in public health, using WHO test kits insecticide-treated filter papers. These insecticides were: deltamethrin with three different concentrations: (0.05%, 0.25% and 0.5%), Bendiocarb 0.1% and Malathion 5%. Bioassays were conducted with 2 to 5 days old non-blood-fed adult female mosquitoes. Each experiment and the controls was replicated three times. The mosquitoes were exposed for a period of one hours and the number of knocked down mosquitoes were recorded after 10, 15, 20, 30, 40, 50 and 60 minutes [14,15]. After exposure, mosquitoes were transferred to insecticide-free observation tubes and maintained with 10% sucrose solution. Final mortality in test and control was recorded 24 hours after exposure. The threshold of susceptibility was fixed at 98% mortality rate for the five active molecules according to the WHO's protocol [16,17].

Data analysis

Susceptibility status of mosquito tested was evaluated in according with the WHO criteria [14], wherein, mortality greater than 98% indicates susceptibility, mortality between 90% and 97% suggests resistance and needs further investigation and mortality lower than 90% confirms insecticide resistance.

The data for each bioassay were collected on adapted sheets and the results compliant and analysed with Excel (Microsoft Office). The percentage of Kd mosquitoes is analysed according to a log-probit model [15]. Kd analysis provides more information than alone mortality and is an earlier indicator of apparition of resistance than mortality. A significant increase in Kd times is all the time observed before a significant decrease in mortality.

Results

Species composition

A total of 693 females belonging to *A. gambiae* s.l. were assayed, in this study. Being 527 to the different insecticides and 166 as control. All the mosquitoes used in the test were preserved in silica gel for future identification by molecular analysis.

WHO susceptibility tests

About 90% of mosquitoes of the mosquitoes exposed was knocked down within an hour of exposure to the chemicals.The results showed that Malathion 5% had the lowest knock-down effect, 68% and tests with deltamethrin 0.25%, 0.5% and bendiocarb 0.1% the highest knock-down effect (Figure 2). For the deltamethrin 0.05% the knock-down effect was about 92% for one hour of exposition.

Our results show the increasing of KDT is more pronounced with the lowest concentration of deltamethrin (0.05%) compared to both deltamethrin 0.25% and 0.5% (Figure 3). Resistance to deltamethrin 0.05% and Malathion 5% as well, was less marked with mortality rates above 80% threshold being observed, suggesting reduced susceptibility which deserves further monitoring in order to establish the phenotypic status of resistance in these vector populations. However, the vector population were find completely susceptible to Deltamethrin 0.25%

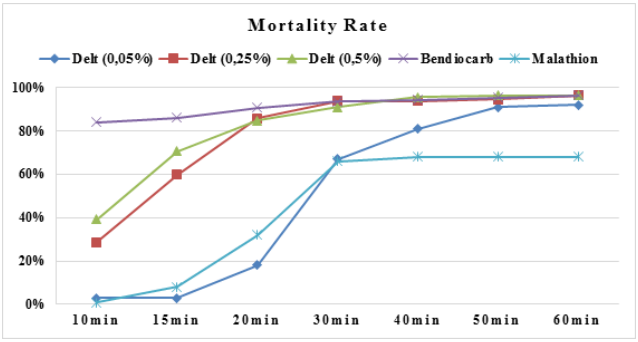

Figure 2: Susceptibility of *Anopheles gambiae* s. l. mosquitoes from Praia, Cabo Verde to insecticides.

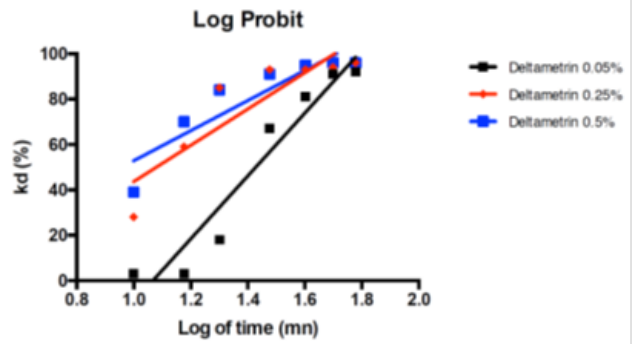

Figure 3: Log Probit analysis of knock-down effect in the *Anopheles gambiae s*. l. in Praia, Cabo Verde.

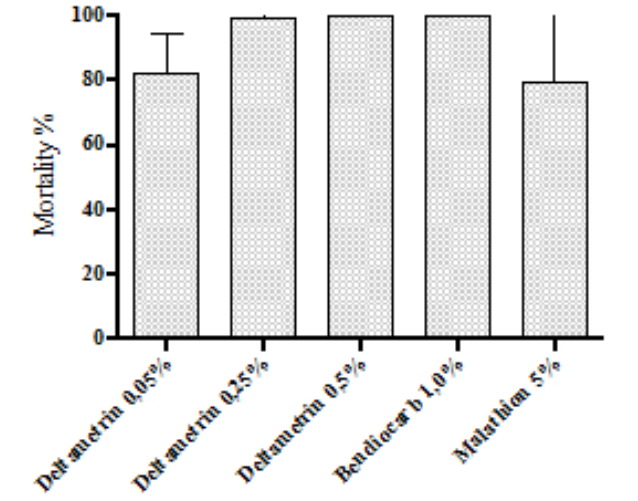

Figure 4: Insecticide mortality rates in *Anopheles gambiae* s.l. populations from study sites of Praia, Cabo Verde, in 2017.

and 0.5% and bendiocarb 1% (mortality=100%) (Figure 4).

Discussion

Anopheles gambiae s.l. was the first species of culicidae identified in Cabo Verde. Also it had been the principal vector of diseases in the country since the colonisation of the islands during the 15th century. During many years, malaria had been the only vectorial disease in the country, with twice interruption in recent history (1968, 1983), what suggest that eliminating malaria from this archipelago is very feasible [18]. In the same time, the data of malaria in the country, show that the disease returns when the effort of control are neglected.

In 1961, with the Global Malaria Eradication Programme, in Cabo Verde operations of Indoor Residual Spraying (IRS) started, with annual spraying campaigns with dichlorodiphenyltrichloroethane (DDT) every six months [18]. With this massive campaign, excellent results in malaria elimination Had been recorded, but there is no data about the impact of the use of this insecticide on public health in the country and the issue of mosquito resistance to this product came years later.

To ensure the success of malaria vector control efforts and malaria elimination, the WHO recommend that the strategic plan, include a comprehensive monitoring and evaluation of resistance [19]. Cabo Verde do not have an insecticide management plan. However, the various normative documents on malaria pre-elimination propose to carry out studies on the bio ecology of vectors, which includes the study of sensitivity/resistance to insecticides [11,20,21].

During this studies, An. gambiae sl was the only species founded in all samplings and used to the tests. The presence of the species in Praia confirm the presence of the vector in the island and the high city and country risk to malaria, which was confirmed by the high cases recorded in 2017, despite the great efforts invested in the process of pre-elimination of the disease in the country. The quantity of larvae in the Praia was not expected, considering the association between the vector density with the rainy season [19], in the year when there was very little rainfall. On the other hand, the presence of the species coincides with the peak of transmission of malaria in the country.

The choice of these insecticides has been dictated by their long-standing use in control operations in Africa and the possibility of having an insecticide of choice or as an alternative molecule in case of occurrence of resistance to deltamethrin. In Cabo Verde the use of deltamethrin date from 1997, as substitute of DDT.

The results of this study provide evidence to deltamethrin 0.05% and malathion 5.0% resistance in Anopheles from Praia. The presence of this resistance to deltamethrin 0.05% is not a surprise taking into account the use of this product for more than 20 years in the country [21], in the fight against disease vectors, both for malaria and for other vector-borne diseases. Another reason could be the pressure of the insecticide, in the sense that it has been used frequently, twice a year, especially after the dengue epidemic registered in the country and 2009 and subsequently the zika epidemic in 2018 [11]. In addition, important to highlight in this context the use DDT and pyrethroids in agriculture for long time in the country. The wide use of these insecticides could had led to a selective pressure in the malaria vector aquatic stages in the country (as notified in the other countries of Wes African, as Burkina Faso and Cote D´Ivoire [22,23]. The strategy of monitoring for vector resistance to insecticides remains a challenge for many malaria endemic countries. The pyrethroid resistance is more common and widespread in major malaria vectors in the world, but resistance to the three other classes commonly used in IRS (organochlorines, organophosphates and carbamates), was confirmed in the last year (2010-2016), in the major malaria vectors. In *Anopheles gambiae* sl, one of the major malaria vector in Africa, the pyrethroid resistance frequency had a moderately increase of 13% during the

period, from 21% to 34% [24]. The results of this study in Praia, Cabo Verde, are in the same sense, with the first appearance of resistance to deltamethrin.

The presence of resistance in anopheles from Praia, is new during the last year, once that sensitivity studies done in 2011 [6], in Anopheles from different municipality in Santiago to the deltamethrin 0.05% and Lambda-Cyhalothrin 0.05% results in total sensitivity of mosquitoes to both insecticides. In the same study KD50 and KD95 were 22 and 48 minutes for deltamethrin and 25 and 56 minutes for Lamda-Cyhalothrin for anopheles populations.

The deltamethrin resistance is reported in the numerous studies of insecticide resistance in West African countries, including neighbouring Senegal [25,26], Southern Mauritania [27], Cote D´Ivoire [28], Mali [29], Burkina Faso and others countries. Hence to be expected in Cape Verde, despite the context of the islands, the geographic location of the country in the West African region.

Some studies suggest two main reasons that support studies about malaria vector resistance. First, because the information about the malaria vector insecticide resistance is essential in decision-making on insecticide choices for IRS. The second important programme issue used in some African countries is the distribution of LLLIN program, a strategy not used in Cabo Verde.

In contrast to some results of other studies carried out in neighbouring countries, namely Senegal [25], An. gambiae sl in Cape Verde is sensitive to bendiocarb and deltamethrin 0.25 and 0.5%. This situation may be explained by not use of bendiocarb in the country to the mosquitoes vector control. The sensitivity of mosquitoes to bendiocarb, with more than 90% mortality in the first 20 minutes of contact, demonstrates that this could be a candidate for substitution of deltamethrin in use in the country, a product in which, the resistance of mosquitoes was detected in this study, despite being in concentration lower.

The sensitivity of the populations to deltamethrin 0.25% and 0.5%, mean that the use of this product have to be used in this concentration and the possibility of a rotation of insecticides in the country, as WHO recommendation. The rotation, mean that removing selection pressure will reverse resistance and that it may therefore be possible at some point to reintroduce the original insecticide into vector control programmes [12]. In according with the same orientation, the changing from IRS with only pyrethroids to a rotation that includes organophosphates and carbamates could increase the cost by approximately 20% and 45% in areas with short and long malaria transmission seasons. It could be a key strategy in the process of eliminating malaria in Cape Verde.

This results, also demonstrate that additional studies must be done in the country and a system of management of sensibility to the insecticide implemented in order to have credible information, to serve as a basis for making the best decisions for the anctivectorial fight, in the process of eliminating malaria in Cape Verde. Studies with additional tests to increase the number of mosquitoes tested and therefore have a much more representative sample size in order to have qualitative data and in more municipalities, especially in Santiago and Boavista, islands with indigenous cases of malaria [3]. The measure the effectiveness of other pyrethroids (permethrin) and other carbamate, organophosphate and organochlorine insecticides which could not be tested due to a lack of sufficient numbers of mosquitoes, that can subsidize the best option in terms of cost-benefit in case of substitution of the insecticide in use in the country. And to determine the dynamics of susceptibility of mosquito populations to insecticides.

To better follow monitoring the Insecticide Resistance Management, select sentinel sites for regular monitoring of vector susceptibility to insecticides for early detection of resistance should be identified, in Santiago or/and Boavista Island and to include this activity in the routine activities of the control program, activity started with the medical entomology lab in the National Institute of Public Health, in the last year.

Conclusion

This study with samples of *An. gambiae sl* from Praia, Cabo Verde, show resistance to the deltamethrin 0.05% and malathion 5.0% and a complete susceptible of the mosquitoes population to deltamethrin 0.25%, deltamethrin 0.5% 0.5% and to bendiocarb 1%. This results are very important to the Nacional Malaria Control Program in the make decision about the choose of insecticides to the vector control in the next years and to decide the implementation of sentinel sites to better monitor the resistance of insecticide in the country. More studies must be done, especially molecular studies, to better understand the mechanisms involved in the process of resistance and to identify the genes mutation and/or metabolic resistance.

References

1. WHO (2017) World Malaria Report. World Health Organization, Geneva, Switzerland.
2. WHO (2015) Global technical strategy for malaria 2016-2030. World Health Organization, Geneva, Switzerland.
3. DePina A, Niang EA, Dia AK, Moreira A, Faye O, et al. (2018) Epidemiological analysis of malaria in Cape Verde, a Country in the pre-elimination phase of the disease. An analysis of malaria data collected from 2010-2016. Malar J 17:236.
4. Ribeiro H, Ramos HC, Capela RA, Pires CA (1980) Os mosquitos de Cabo Verde (Diptera: Culicidae) – sistemática, distribuição, bioecologia e importância médica. Junta de Investigações Científicas do Ultramar, Lisboa.
5. Cambournac FC, Petrarca V, Coluzzi M (1982) *Anopheles arabiensis* in Cape Verde archipelago. Parassitologia 14: 265- 267.
6. Dia I, Alves J, de Pina AJ, Rodrigues J, Leite C (2011) Mission d'appui sur l'étude de la bio-écologie et la sensibilité aux insecticides des vecteurs du paludisme au Cap-Vert. World Health Organisation, Geneva, Switzerland.
7. Alves J, Gomes B, Rodrigues R, Silva J, Arez AP, et al. (2010) Mosquito fauna on the Cape Verde Islands (West Africa): Un update on species distribution and a new finding. J Vector Ecol 35: 307- 312.
8. Coulibaly A (2005) Mission de consultation entomologique au Cap Vert. Rapport de mission OMS. World Health Organization, Geneva, Swizterland.
9. Diallo M (2003) Mission de consultation des vecteurs du paludisme dans l'île de Santiago, Republique du Cap-Vert, Praia ; Ministère de la Santé.
10. Pinto J, Sousa CA, Arez AP, Alves J, Modiano D, et al. (1999) Assessment of malaria transmission in an area with very low mosquito density. Res Rev Parasitol 59: 23-26.
11. MS (2015) Manual de Luta Integrada de Vectores; Programa Integrado de Luta Contra as Doenças Transmitidas por Vetores e Problemas da Saúde Associados ao Meio Ambiente. Ministério da Saúde, Cabo Verde.
12. INE (2013) Projecção Demográficas de Cabo Verde, 2010-2030, Cabo Verde. Instituto Nacional da Estatística de Cabo Verde. Weatherspark.com. Condições meteorológicas médias de Cidade da Praia Cabo Verde,
13. WHO (2016) Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. World Health Organization, Geneva, Swizterland.
14. Finney DJ (1971) Probit Analysis. Cambridge University Press, New York, USA.

15. WHO (2012) Global Plan for Insecticide Resistance Management in Malaria Vectors. World Health Organization, Geneva, Switzerland.

16. WHO (2013) Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. World Health Organization, Geneva, Swizterland.

17. Ministry of Health (2012) Eliminating malaria: Moving towards sustainable elimination in Cape Verde. Ministry of Health Cape Verde and the World Health Organization and the University of California, San Francisco.

18. World Health Organization (2009) Global Malaria Action Plan World Health Organization. World Health Organization, Geneva, Swizterland.

19. MS (2014) Plano Estratégico de Pré-Eliminação do Paludismo em Cabo Verde, 2014-2017. Ministério da Saúde, Cabo Verde.

20. WHO (2013) Relatório Geral da Revisão da Performance do Programa Nacional de Luta contra o Paludismo. Ministério da Saúde, Cabo Verde.

21. Diabaté A, Baldet T, Chandre F, Akogbeto M, Guiguemde TR, et al. (2002) The role of agricultural use of insecticides in resistance to pyrethroids in *Anopheles gambiae s.l.* in Burkina Faso. Am J Trop Med Hyg 67: 617-622.

22. Chandre F, Darriet F, Manguin S, Brengues C, Carnevale P, et al. (1999) Pyrethroid cross-resistance spectrum among population of *An. gambiae s.s.* from Côte d'Ivoire. J Am Mosq Control Assoc 15:53-9.

23. WHO (2008) Global report on insecticide resistance in malaria vectors: 2010-2016; World Health Organization, Geneva, Swizterland.

24. Dia AK, Guèye OK, Niang EA, Diédhiou S, Sy MD, et al. (2018) Insecticide resistance in *Anopheles arabiensis* populations from Dakar and its suburbs: Role of target site and metabolic resistance mechanisms. Malaria J 17:116.

25. Niang EA, Konaté L, Diallo M, Faye O, Dia I (2016) Patterns of insecticide resistance and knock down resistance (kdr) in malaria vectors *An. arabiensis, An. coluzzii and An. gambiae* from sympatric areas in Senegal. Parasit Vectors 9:71.

26. Lemrabott MA, Salem MA, Brahim KO, Brengues C, Rossignol M, et al. (2018) Seasonal abundance, blood meal sources and insecticide susceptibility in major anopheline malaria vectors from southern Mauritania. Parasit Vectors 11:232.

27. Zoh DD, Alou LP, Toure M, Pennetier C, Camara S, et al. (2018) The current insecticide resistance status of *Anopheles gambiae* (s.l.) (Culicidae) in rural and urban areas of Bouaké, Côte d'Ivoire. Parasit Vectors 11:118.

28. Cisse MB, Keita C, Dicko A, Dengela D, Coleman J, et al. (2015) Characterizing the insecticide resistance of *Anopheles gambiae* in Mali. Malaria J 14:327

29. Namountougou M, Simard F, Baldet T, Diabaté A, Ouédraogo JB, et al. (2012) Multiple insecticide resistance in *Anopheles gambiae s.l.* populations from Burkina Faso, West Africa. PLoS ONE 7: e48412.

Author Affiliations

Top

[1]*Programa de Pré-Eliminação do Paludismo, CCS-SIDA, Ministério da Saúde e da Segurança Social, Cabo Verde*

[2]*Ecole Doctorale des Sciences de la Vie, de la Santé et de l´Environnement (ED-SEV), Université Cheikh Anta Diop (UCAD) de Dakar, Sénégal*

[3]*Laboratório de Entomologia Médica, Instituto Nacional de Saúde Pública, Cabo Verde*

[4]*UPB/Institut de Recherche en Sciences de la Santé, Centre Muraz, Bobo-Dioulasso, Burkina Faso.*

[5]*Programa Nacional de Luta contra o Paludismo, Ministério da Saúde e da Segurança Social, Cabo Verde*

[6]*Laboratoire d'Ecologie Vectorielle et Parasitaire, Faculté des Sciences et Techniques, Université Cheikh Anta Diop (UCAD) de Dakar, Sénégal*