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Susceptibility status of Culicine and Anopheline mosquitos towards different classes of insecticides at different concentration used for IRS and LLINs in the Gash Barka zone, Eritrea

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Abstract
Background: Resistance in *Anopheles* mosquitoes to at least one class of insecticides is reported in 90% of malaria-endemic countries. There has been a rapid emergence in insecticide resistance among mosquito population to commonly used public health insecticides. This trend of rapid increase in observed insecticide resistance following exposures is alarming as it reduces our overall chemical arsenal to control disease vectors and the efficacy of many vector control products that have been and will be deployed. Furthermore; there is limited information on insecticide susceptibility status of human-biting mosquitoes in Eritrea. This study aimed to determine insecticide susceptibility status of human biting mosquitoes in an urban area of western Eritrea.
Methods: The study was conducted in Tesseney district, Eritrea in which the Entomology laboratory center located. Insecticide susceptibility bioassays were performed according to the World Health Organization standard operating procedures on four days old human biting mosquitoes. Each mosquito was exposed to three classes of insecticides at different concentrations commonly used for IRS & LLINs. 1-hour knockdown and 24 hours' mortality rates (%) post insecticide exposure were determined.
Results: Mosquito type tested were belongs to *Anopheles, culex and Aedes.* Aedes mosquitoes were susceptible to all tested insecticides except to pirimiphosmethyl (0.25%). Anopheles mosquitoes has found resistant to Pirimiphosmethyl (0.25%), Alpha cypermethrine (0.05), possible resistance to Alpha cypermethrine (0.25%, 0.5%)and bendiocarb 0.1%, but showed susceptible to Pirimiphosmethyl (1.25%, 2.25%) bendiocarb (0.5%,1%). our finding shown, culex mosquitoes were resistant to all tested insecticides except susceptible with Pirimiphosmethyl (2.25%) and Alpha cypermethrine (0.5) and possible resistance to Alpha cypermethrine (0.25%).
Conclusion: Our results revealed that different type of mosquitoes had different susceptibility status to different class of Insecticide commonly used for IRS and LLINs in our area. The current susceptibility status of the tested mosquitoes showing the need to select the most efficacious insecticide for the least susceptible mosquito species to achieve successful mosquito control.

Keywords: Insecticide resistance, mosquitoes, susceptibility, Eritrea

Introduction
Mosquito-borne diseases remain the major cause of illness and deaths in many parts of the world, particularly in tropical and sub-tropical climates [1]. Mosquito -borne diseases such as dengue, Zika, leishmaniasis, Lyme disease, yellow fever and malaria account for more than 700,000 deaths annually [2]. Several mosquito-borne diseases, including dengue, Zika, chikungunya, and West Nile virus, have (re) emerged over the decade [3], which has led to an increase in morbidity and mortality [4]. To illustrate, the Zika outbreak in 2016 infected more than 130,000 people in Brazil alone [5], and there are an estimated 96 million cases of dengue annually, with more than 3.9 billion people in over 128 countries at risk of contracting the disease [2]. Moreover, malaria still kills an estimated 405,000 people annually, and half of the world's population is thought to be at risk. Despite intensified malaria control and elimination efforts over the past two decades, it has been 5 years without significant reduction in the number of malaria cases globally.

Therefore, and because of the current impact of COVID-19 pandemic on those efforts, the targets outlined in World Health Organization (WHO)'s Global Technical Strategy for Malaria 2016-2030 are unlikely to be met [6, 7].

Over the past two decades, successful implementation of various strategies to combat malaria has led to a significant decrease in malaria incidence in Eritrea [8-12]. In 2012, there was 89% reduction in malaria incidence from 157 cases/1000 populations at risk in 1998 to 17 cases/1000 in 2016. In the same year, there was a 98% reduction (0.004 deaths/1000) in malaria-specific deaths compared to 0.198 deaths/1000 in 1998. In some parts of Eritrea, there seems to be a "break in malaria transmission" as subzones that previously reported thousands of cases are reporting very few or nil cases. Furthermore, the NMCP has developed National Malaria Strategic Plan to eliminate malaria in Eritrea by 2030 [13].

A short-term goal of the Global Plan for Insecticide Resistance Management (GPIRM) in malaria vectors, developed in 2012 by the WHO, is to preserve the effectiveness of current vector control interventions [14]. One of the key pillars of the strategic framework for Global Technical Strategy for malaria 2016-2030 (GTS) is to ensure universal access to core malaria interventions. Indoor residual spraying (IRS) and insecticide-treated nets remain the primary vector control tools in the GTS [15]. These two control interventions have accounted for almost 60% of global investment in malaria control in recent times [16]. Globally, malaria interventions through vector control and effective treatment have reduced malaria mortality by 62% between 2000 and 2015 [17].

With no (Prophylactic) drugs and/or vaccines available to prevent or control many vector-borne infectious diseases, chemical vector control remains a cornerstone in disease control and prevention [18]. Insecticides are widely used in public health to reduce vector populations. Interventions include chemical fogging to control *Culex* ssp. (vectors of, e.g., West Nile virus and lymphatic filariasis) and *Aedes* ssp. (vectors of, e.g., dengue, yellow fever, and Zika), and indoor residual spraying (IRS) and long-lasting insecticidal nets (LLINs) for malaria control [19]. The US President's Malaria Initiative supported IRS in over 5.7 million houses in 2018 [20], and over 1.8 billion pyrethroids-based bed nets have been distributed between 2010 and early 2020, of which close to 1.6 billion were distributed in sub-Saharan Africa [21]. This unprecedented quantity of insecticides exerts an exceptionally strong selective pressure for resistance and, unsurprisingly, insecticide resistance to most of the WHO-approved public health insecticides has now been reported around the world [22].

Twelve insecticides recommended by WHO for IRS currently, which belong to four chemical groups including one organochlorine, six pyrethroids, three organophosphates and two carbamates [23, 24]. DDT resistance in the adult of *Aedes aegypti*, *Ae. albopictus* and susceptibility to Temephos, *Bacillus thuringiensis* and metabolic resistance of the current species to deltamethrine and DDT have been reported in Africa [25]. Resistance of *Ae. aegypti* larvae to Temephos has been reported in Asia [26, 27]. In addition, larval resistance of *Aedes albopictus* to Temephos have been reported in Malaysia [28], Thailand [29]. Adult susceptibility test on *Ae. aegypti* against some pyrethroids has been reported in various research study [29-32]. In spite of some reports due to resistance of *An. stephensi* against DDT, Dieldrin and Malathion in Iran [33, 34]. Mechanism of resistance of *An. stephensi* against Temephos has been reported by [35]. For malaria vectors specifically, resistance in *Anopheles* mosquitoes to at least one class of insecticides is reported in 90% of malaria-endemic countries, and 32% of the countries have reported resistance to the four classes of insecticides (Pyrethroids, carbamates, organophosphates, and organochlorines) that were recommended until 2016 [36]. Resistance to clothianidin, the latest approved active ingredient that is used in several IRS products and considered the silver bullet in insecticide-resistance management, has now been reported in Central Africa as well [37]. This trend of rapid increase in observed insecticide resistance following exposures is alarming as it reduces our overall chemical arsenal to control disease vectors and the efficacy of many vector control products that have been and will be deployed. To adequately manage insecticide resistance, to develop effective vector control strategies, and to understand the role of insecticide resistance in recently reduced success of malaria eradication, close monitoring of the insecticide susceptibility status in vector populations is critical. Insecticide susceptibility is typically assessed via WHO tube tests or CDC bottle bioassays for adulticides and larval bioassays for larvicides. These different tests, used around the world by researchers, non-governmental organizations (NGOs), and national and local governments, do not replicate well, are not comparable, and maybe more importantly do not necessarily predict if the observed resistance does actually translate to a loss in efficacy of actual vector control product and thus control failure.

In Eritrea insecticide resistance is well established with *An. gambaie complex s.l* main malaria vector being resistant to DDT, Permethrin and *lambda*cyhalothrine. However, no enough evidence for the susceptibility status of other mosquito vectors rather than Anopheline available in the country such as Aedies and culex spps for different class of insecticide used for adult control methods (IRS& LLINs). The NMCP in Eritrea currently uses both carbamates, organophosphates in rotation for IRS and pyrethroids for LLINs to control adult mosquitos in conjunction with routine vector control (VC) operations that includes larviciding using Temephos (organophosphates) and environmental management to reduce aquatic stage of mosquito through elimination of breeding habitats of mosquito. Based on the current malaria situational analysis country wide there is an increase of malaria epidemiology from the year 2019 onwards in Gash Barka region it accounts for about 80% of the total burden of the country. These could be due to insecticides resistance pattern of the local mosquitoes to the used insecticides, therefore few is known on resistance status of the available WHO Impregnated papers and no publication was done previously. Therefore, an intensive study on insecticide resistance and susceptibility of the species of human biting mosquito is needed.

General objective

To determine Susceptibility status of Culicine and Anopheline mosquitos towards different classes of insecticides (Bendiocarb, Pirimiphosmethyl, and Alphacypermethrine) at different concentration used in the Gash Barka zone in the year 2020.

Specific objectives

- To determine the presence of resistance in different mosquito's population (Anopheles, Culex, Aedes).
- To identify the strength of the different classes of insecticides towards different types of mosquitoes.
- To identify the strength of the same insecticide at different concentration towards different type of mosquitoes.

Material and Methods

Study site

Gash Barka region (GBZ) is one of the six zone of the country it has an estimated total population of 1,020242. It is the main source of economic of the nation having many dams and macro dams used for agricultural project and different mining sites. The populations of the zone depend mainly on agriculture, small trading and animal husbandry. It has 16 districts which are named as sub zones. It is the highest malarious zone, it accounts for about 88% of the total burden of the country $DHIS_2$ report of (2020). Out of the total population 95 % (969, 546) of the population are at risk of malaria and the situation of malaria is heterogeneously distributed. It has rain period of April to May and followed summer rains start on June and to September with an average annual rainfall of 392 mm distributed over the months of the rainy-season. The area is characterized by minimum temperature 16.9 ^{0}C and maximum of 40.0 ^{0}C with relative humidity (RH) ranges from 38-72% distributed over the months according to the data obtained from the ministry of agriculture (2019).

This cross- sectional study was conducted between August and December 2020 in one of the district Gash Barka region namely Tesseney district in which the Entomology laboratory center located. The study area is one of highest malaria sub zones and it has high distribution of different mosquito vectors (Anopheline and Culicine). The district has a total population of 92, 646, it is situated in western part of the region from center (Barentu town) and the economic income depends mostly on farming and animal raising, and small part of them are merchants, it is also known as land port with Sudan. Gash River is permanent breeding site which affect the district on malaria incidence. It lies at latitude 15^{0}6'53" N and longitude 36^{0}39'46" E with elevation of 610m above sea level (Using GPS). The intervention used to halt or reduce malaria is source reduction through filling and drained followed by application of Temephos and BTI for the mosquito larval habitats which are not convent for elimination and human use. Organophosphates and Carbamates are used for IRS in rotation every two years to reduce resistance pattern. Pyrethroids are only class of insecticide used of LLINs. The study site has experienced different out breaks of Malaria, Dengue fever and Chikungunya for years.

Mosquito collection and identification

Mosquito larvae were collected from the wild in order to obtain an age-standardized sample of the adult population for the insecticide bioassay tests. Mosquito larvae were collected by using a standard dipper from natural habitats and man-made habitats *e.g.* standing water bodies and animal foot print, puddles, water containers, barrels, pots, discarded tires at Tesseney town. Collected larvae were transported and sorted in entomology laboratory to remove larval predators. The collected larvae were identified physically as *Anopheles*, *Culex*, and A*edes* species based on the following characters (On siphon, swimming character, and resting character) and kept at different tray and were kept in separate larval rearing containers basing on their larval stages. Emerged pupae were sucked from the larval containers using a plastic pipette and placed in plastic cups inside the mosquito cages to prevent emerging adult mosquitoes from escaping. Emerged adult mosquitoes were kept in the cage for 2- 5 days and feed on sugar.

Insecticide susceptibility bioassays

Insecticide susceptibility bioassays were performed according to standard WHO guidelines (WHO, 2013). Assays were conducted with three insecticides at different concentrations namely, bendiocarb (0.1%, 0.5%, 1%), Alphacypermethrine (0.05%, 0.25%, 0.5%), and pirimiphosmethyl (0.25%, 1.25%, 2.5%). for each concentration of insecticides four exposure and two control tubes was prepared. 20-25 adult mosquitos from each species were transferred separately to each test tube for each concentration of insecticides. In our case the susceptibility testing was conducted using only female mosquitoes aged 3-5 days that non-blood fed only fed on sugar. Tested mosquitoes were monitored at different time interval of 10, 15, 20, 30, 40, 50 and 60 minutes. Anopheles, Culex and Aedes mosquitoes were tested against the three (3) class insecticides at different concentration belonging to the major public health insecticide classes. For all tested mosquitoes knocked-down were recorded after 60 min exposure to the insecticide. After exposure time, all mosquitoes were transferred to the holding tubes and provided with 10% glucose solution through a cotton wool. The mortality rates were determined at 24 hours post exposure. In each bioassay, a control experiment using papers impregnated only with insecticide carrier oil was performed in the same way as in treatment experiments. Susceptibility tests were conducted in the entomology laboratory under 27±20C and 75-±5% temperature and humidity, respectively. Dead and surviving mosquitoes at the end of an experiment were kept in separate Eppendorf tubes containing silica gel and labeled [38].

Data analysis

The knockdown (KD) mosquito data was subjected to Polo Plus probit and log it analysis version 1, 2002-2009 LeOra Software to estimate the KDT50 and KDT95, which is the time taken to knock down 50% and 95% of the exposed mosquitoes, as well as their 95% confidence interval. The 24hr mortality rate (%) was established by counting the number of mosquitoes killed at the end of the holding period (24 hours) divided by the total number of mosquito exposed times 100. The insecticide susceptibility status of tested mosquitoes was assessed based on standard guidelines (WHO, 2013). Mortality range 98-100% indicates susceptible mosquito population; 90-97% suggests possible resistance that needs to be confirmed and below 90% indicates existence of resistance. When control mortality was between 5 and 20%, the mortality was corrected using the Abotts formula [38]. Percent corrected was computed using Abbott's Formula.

$$\%\ \text{corrected mortality} = \frac{\%\ \text{Kill in treated - }\%\ \text{Kill in control}}{100\%\ \text{-}\%\ \text{Kill in control}} \times 100$$

If the control mortality was > 20%, the test was repeated. If the mortality was < 5%, the test was considered valid and no correction was needed.

Results

A total of 2242 mosquitoes comprising 714 (31.84%) Anopheles, 738 (32.91%) Culex and 790 (35.23%) were tested to three insecticides in different concentration in order to assess the susceptibility status. And also 350 Anopheles, 369 Culex and 374 Aedes mosquitoes were served as control. The result of the efficacies of the three chemical ingredients namely, permethrin, propoxur, and malathion from selected three main classes of insecticides respectively, tested against the adult female An. Gambiae, *Ae. aegypti* and *Cx. quinquefasciatus* sample collected from the Tesseney town shown in (table-1-3). Insecticide susceptibility levels in the mosquitoes Varied from resistant to susceptible. In each insecticide tested, the adult bioassay revealed considerable variations in susceptibility status of the adult female An. gambiae mosquitoes after 1hr/60 minutes' exposure and the 24hr.

Indeed, vector control interventions such as long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS) have been widely used. Our findings revealed the spread of insecticide resistance in the local mosquito population. However, the three mosquito species also showed various resistance to some tested insecticides. Therefore, it is evident that the evaluation and selection of the most efficacious compound for the least susceptible mosquito species is an important step for effective mosquito control. The 24hr mortality rate post-exposure to insecticides revealed resistant of Anopheles mosquitoes to Pirimiphosmethyl (0.25%), Alpha cypermethrine (0.05) with mortality of 81.3% and 85.8% respectively, possible resistance to Alpha cypermethrine (0.25% and 0.5%) and bendiocarb 0.1%with mortality 93% & 95.8%,92.99% respectively. Aedes mosquitoes were susceptible to all tested insecticides except to pirimiphosmethyl (0.25%) resistant with mortality 71.7%. Unlike the others, culex mosquitoes were resistant to all tested insecticides except to Pirimiphosmethyl (2.25%) and Alpha cypermethrine (0.5) susceptible with mortality 100% and possible resistance to Alpha cypermethrine (0.25%) with mortality 91.6%.

Table 1: Insecticide resistance status of mosquito species exposed to pirimiphosmethyl at different concentrations

Species	Pirimiphosmethyl0.25%		Pirimiphosmethyl 1.25%		Pirimiphosmethyl 2.25%	
	Mortality %	Resistance status	Mortality %	Resistance status	Mortality %	Resistance status
Anopheles	81.3%	R	100%	S	100%	S
Culex	22.7%	R	83.3%	R	100%	S
Aedes	71.7%	R	100%	S	100%	S

Table 2: Insecticide resistance status of mosquito species exposed to alpha cypermethrine at different concentrations

Species	Alphacypermethrine 0.05%		Alphacypermethrine 0.25%		Alphacypermethrine 0.5%	
	Mortality %	Resistance status		Mortality %	Resistance status	
Anopheles	85.8%	R	93.2%	PR	95.8	PR
Culex	80.4%	R	91.6%	PR	100%	S
Aedes	98.9%	S	100%	S	100%	S

Table 3: Insecticide resistance status of mosquito species exposed to bendiocarb at different concentrations

Species	Bendiocarb 0.1%		Bendiocarb 0.5%		Bendiocarb 1%	
	Mortality %	Resistance status	Mortality %	Resistance status	Mortality %	Resistance status
Anopheles	92.99%	PR	97.42%	S	100%	S
Culex	26.3%	R	27.4%	R	73.1%	R
Aedes	100%	S	100%	S	100%	S

RS=Resistance status; R=resistance, S=susceptible and PR= Possible resistance

Discussion

Management of insecticide resistance in disease vectors relies on detailed and frequent resistance monitoring. Also other Entomological data are essential to build a relevant strategic plan for prevention of vector borne disease. The NMCP is working in close collaboration with stakeholders and research institutions to build on the best evidence-based strategic plan to fight Mosquito borne disease. This study focused on *An. gambiae* (s.l.), *Ae. aegypti*, *Cx. Quinquefasciatus which are* the important vectors of Malaria, dengue fever and filariasis respectively.

In the present study, *An. gambiae* s.l. belongs to genus Anopheles were found to be susceptible to bendiocarb at the concentration of 0.5% & 1%. However, it showed that possible resistant to bendiocarb at 0.1%. Similar study conducted in the same area (Tesseney district) *An. gambiae* s.l found to be susceptible to bendiocarb at the concentration of 0.1% & 1% [39]. This is in contrary to previous findings in Tanzania [40, 41] which reported the species to be susceptible to bendiocarb 0.1%. In this study, *An. gambiae* s.l. was found to be susceptible to pirimiphosmethyl at concentration 1.25% & 2.25%. This study is agreed with previous study conducted in the same area (Tesseney district) which reported *An. gambiae* s.l to be susceptible to pirimiphosmethyl at concentration 1.25% &2.25% [39]. However, our finding shown that it is resistant to pirimiphosmethyl at concentration 0.25%. Our findings contrary with results from previous studies in Muleba, Tanzania [42] and Atacora, Benin [43] which reported the species to be susceptible to pirimiphosmethyl at concentration 0.25%. In Eritrea Pirimiphosmethyl formulation is effective and appropriate insecticide for malaria prevention. Zambia and Zanzibar also reported that Pirimiphosmethyl formulation has highly effective and appropriate insecticide for IRS and can be used for management of insecticide resistance in malaria vectors [44, 45].

The present study has shown that, *An. gambiae* s.l is possible resistant to Alpha cypermethrine at concentration of 0.25% and 0.5% and resistant at concentration of 0.05%. In previous studies in Malawi and Kwazulu-Natal, South Africa *An. funestus* showed high resistance to pyrethroids insecticides [46, 47]. In Kwazulu-Natal, *An. funestus* resistance to deltamethrine used for IRS, led to an increase in malaria incidence to over six-folds in during late 1990s [48].

In Eritrea resistance status of *Cx. quinquefasciatus* demonstrated for the first time in the study area. *Cx. quinquefasciatus*. Is important vector of e.g., West Nile virus and lymphatic filariasis) In the area of study, Tesseney district *Cx. quinquefasciatus* belongs to genus of culex were tested to the three class of insecticides at different concentrations. culex larvae were collected from both natural breeding habitat (*e.g.* standing water bodies, and animal foot print, puddles, water containers, barrels) at Tesseney town. Our findings have shown that, *Cx. quinquefasciatus* is highly resistant against majority of classes of insecticides at all concentrations tested. Multiple resistances to deltamethrine, permethrin, malathion, fenitrothion, propoxur, DDT, chlorpyriphos and lambdacyhalothrine have been reported in *Cx. quinquefasciatus* in Brazil [49, 50], Wete Island in Tanzania [51] and India [52]. In a situation where resistance is very high, other control measures such as environmental management such source reduction and selection of effective compounds can be opted for successful mosquito control. The present study has found that, *Cx. quinquefasciatus* was susceptible to pirimiphosmethyl at concentration 2.25%. Our findings concur with the stud conducted in India [53]. Also our study shown that *Cx. quinquefasciatus is susceptible* to Alpha cypermethrine at concentration 0.5%.

In Eritrea resistance status of *Ae. aegypti* conducted for the first time in the study area. In the area of study, Tesseney district only *Ae. aegypti* species belongs to genus of Aedes were found. The Aedes aegypti larva were collected from the artificial (man-made) breeding habitat like discarded tires, cement water container, metal water containers, barrels, pots. *Ae. aegypti* is an important vector of, e.g., dengue, yellow fever, chikungunya and Zika. During the study period Aedes aegypti were tested to the three class of insecticide at different concentration in the study area. The present study showed that Ae. Aegypti was susceptible to bendiocarb at all concentration that were tested 0.1%, 0.5%, 1%. Ae. Aegypti showed susceptible Alphacypermethrine at all concentration that were tested 0.05%, 0.25%, 0.5% and also susceptible to pirimiphosmethyl at concentration 1.25% & 2.25%. However, in the study area *Ae. aegypti* showed resistance to pirimiphosmethyl at the concentration 0.25%. similar study conducted in Brazil where *Ae. aegypti* was reported to be resistant to Temephos which is an organophosphate [54]. Temephos resistance may occur due to alterations in the target site of the insecticide; the acetylcholinesterase gene or through elevated levels or differential efficacy of metabolic genes [54].

The present study has revealed different levels of insecticide susceptibility status to three classes of commonly used insecticides in the most common mosquito vectors of human diseases in western part Eritrea. The study has also provided baseline information on the insecticides susceptibility status of Ae. aegypti & *Cx. quinquefasciatus* and the current susceptibility status of *An. gambaie s.l* in the study area. The findings of the present study call for immediate insecticide resistance management for wisely mosquito borne disease prevention.

Conclusion

In summary, we evaluated the resistance status of three class of insecticide at different concentration with different modes of action against adult Anopheles, Culex & *Aedes*. Our results revealed that different type of mosquitoes had different susceptibility status to different class of Insecticide commonly used for IRS and LLINs in our area. The current susceptibility status of the tested mosquitoes showing the need to select the most efficacious insecticide for the least susceptible mosquito species to achieve successful mosquito control. There is need therefore to opt for other more effective control methods, including larval source management and larviciding for this group of mosquitoes. The study has also provided baseline information on the insecticides susceptibility status of non-malaria mosquito vectors

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