INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 22, No. 1, p. 8-17, 2023

RESEARCH PAPER OPEN ACCESS

Anopheles stephensi: The emerging vector of malaria in the Republic of Djibouti, Horn of Africa

Renaud Govoetchan[*1,2,3], Mohamed Mousse Ibrahim[4], Arthur Sovi[1,2,3], Houssein Mouhamed Omar[5], Abdillahi Omar Boulhan[5], Houssein Youssouf Darar[4]

[1]*Centre for Research in Entomology of Cotonou, Cotonou, Benin*

[2]*Faculty of Agronomy, University of Parakou, Parakou, Benin*

[3]*Faculty of Infectious and Tropical Diseases, Disease Control Department, London School of Hygiene and Tropical Medicine, London, United Kingdom*

[4]*National Public Health Institute, Djibouti-ville, Djibouti*

[5]*National Malaria Control Programme, Djibouti-ville, Djibouti*

Key words: Djibouti, *Anopheles stephensi, Anopheles gambiae,* Insecticide resistance

http://dx.doi.org/10.12692/ijb/22.1.8-17 Article published on January 01, 2023

Abstract

The present study investigated mosquito species composition and phenotypic insecticide resistance profile to support decision-making in vector control in the Republic of Djibouti at the Horn of Africa. Adult mosquitoes were collected between December 2016 and December 2017 across 20 sentinel sites established in the 6 regions of the country using both Centers for Disease Control (CDC) miniature light traps and pyrethrum spray catches (PSC). Female mosquitoes were kept aside, for morphological identification to species by an expert entomologist using appropriate taxonomic keys by Gillies & Coetzee and Glick. Bioassays were also conducted in *An. stephensi* from Djibouti-ville against nine insecticides used in public health. A total number of 12,538 host-seeking mosquitoes belonging to four genera (*Anopheles, Culex, Aedes, Uranotaenia*) comprising 12 species were collected. Among these, *A. gambiae* S.L. and *A. stephensi* were the two major malaria vectors identified while secondary malaria vectors such as *A. nili somalicus*, *A. dthali* and *A. azaniae* were also collected. *Culex quinquefasciatus* was the most abundant mosquito species in the 6 regions. WHO susceptibility tests performed on *A. stephensi* population from Djibouti-ville showed resistance to pyrethroids, organophosphates, carbamates and DDT. The resistance intensity bioassays indicated low to moderate intensity of resistance with pyrethroid insecticides and the organophosphate pirimiphos methyl. Meanwhile pre-exposure to PBO suggested involvement of P450 detoxification enzymes in pyrethroid resistance. These findings revealed the urgent need to develop and implement a programme for monitoring and managing insecticide resistance in local vector populations with efficient control strategies in Djibouti.

* **Corresponding Author:** Renaud Govoetchan ✉ renaud292@yahoo.fr

Introduction

Located in the Horn of Africa, the Republic of Djibouti shares borders with Eritrea, Ethiopia and Somalia and has just over 300 km of coastline along the Red Sea and Gulf of Aden (Hatem, 1996). With an area of 23,200km² consisting mainly of plateau, plains and highlands, the Republic of Djibouti has a hot and humid climate, with a temperature that varies between 30°C in January and 43°C in July with an average humidity of 69% (Schulman, 2019). Rainfall is rare (<130mm annually), however unusual rains may occur across the country causing heavy rainfall and flooding in residential areas of the capital Djibouti-ville (Schulman, 2019).

In the Republic of Djibouti, mosquito-borne diseases are primarily transmitted by malaria vectors and secondarily dengue fever vectors, followed by other diseases with suspected cases such as West Nile virus and Leishmaniasis (Faulde *et al.,* 2012; Rodier, 1995). While the Horn of Africa is known to be highly susceptible to mosquito-borne diseases, the Republic of Djibouti was historically thought to be a malaria meso to hypo-endemic country with intermittent epidemics (Rodier, 1995). However local populations have experienced only low and unstable malaria transmission and intermittent epidemics (Fox *et al.,* 1991; Fox *et al.,* 1989) with most of cases detected in people returning from neighbouring countries (Khaireh *et al.,* 2013; Crowell *et al.,* 2012).

The first case of malaria was reported at the very beginning of the 20th century, but it was not common until 1984 that malaria became a public health problem after a significant number of imported malaria cases were recorded. Since then, malaria cases have continued to increase, most importantly in Djibouti-ville. From 1988, there was an increase in malaria cases throughout the country, even in the regions of Tadjourah and Obock that were not affected in the past. In order to address the progression of the disease, the country implemented its first vector control programs based initially on larval control (Louis, 1988; Carteron *et al.,* 1979) strengthened later with the introduction of insecticide-treated bed nets.

Unfortunately, investments and control efforts did not commensurate with the risks of disease, and major malaria outbreaks were reported. In 1991, a record of 7,338 microscopically confirmed malaria cases declining to 4,770 cases in 1993 (Rodier, 1995).

Contrastingly, an unusual increase of malaria cases was reported in 1999 in and around Djibouti-ville due to the emergence of chloroquine resistance (Rogier *et al.,* 2011). The cases detected were mainly caused by *Plasmodium falciparum* but also *P. vivax* which was responsible of 3% of malaria burden (UNDP, 2013). The last uncommon urban outbreak of malaria was observed in 2013 with 1228 reported cases of which 83% were from Djibouti-ville alone (UNDP, 2013).

The entomological surveillance in Republic of Djibouti was not a routine exercise and as such the monitoring programme at country level is very poor. Most of the existing data were generated as part of the international European Union Naval Force Somalia mission “Atalanta” (Faulde *et al.,* 2012; Faulde & Ahmed, 2010) by the country's military partners that have troops based at various locations throughout the territory. The local malaria transmission was attributed to *Anopheles arabiensis* in all the 6 regions of the country (WHO, 2012).

However, a larval survey has reported the presence of *A. nili* in Ali Sabieh, southern region of Djibouti. More recently, *A. stephensi*, the Asian malaria mosquito vector of *Plasmodium falciparum* and *P. vivax* was incriminated in the outbreak which occurred in 2013 (Faulde *et al.,* 2014).

The current study updates malaria entomological data mandatory for decision making on control strategies implementation. The data was generated over a recent entomological surveillance conducted between December 2016 and December 2017, in 20 sentinel sites located along the south-north transect of Djibouti Republic. Mosquito species compositions, as well as insecticide resistance status of the main malaria vector identified in the Djibouti-ville were assessed to support decision-making for the implementation of an efficient control program.

Materials and methods

Study sites

The mosquito surveys were conducted over three collection time points (December 2016, March/April 2017 and October/November 2017), in the six regions (Djibouti-ville, Arta, Ali-Sabieh, Dikhil, Tadjourah and Obock) of the Republic of Djibouti (Fig. 1). Overall, 20 sentinel sites established by the National Malaria Control Program were surveyed. The geographical position of these collection sites in the administrative regions is summarized in Table 1 below.

Table 1. Sentinel sites defined by the National Malaria Control Programme in Djibouti.

N°	Region	Sub-region/location	
1	Djibouti-ville	Ras Dika	Port Sheraton
		Boulaos	Ambouli Arhiba Einguella Farhad
		Balbala	Balbala I Balbala II Hayableh PK12
2	Arta	Arta-ville Damerjog	
3	Ali Sabieh	Ali Sabieh-ville Ali Addé	
4	Dikhil	Dikhil-ville Mouloud	
5	Tadjourah	Tadjourah-ville Kalaf	
6	Obock	Obock-ville Markazi	

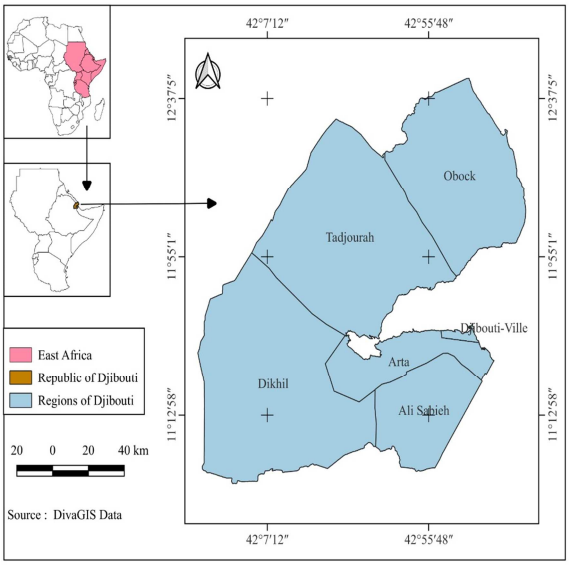

Fig. 1. Map showing the 6 regions of Djibouti Republic.

Mosquito species composition

Sampling methods

Over each of the three collection rounds undertaken across the sentinel sites, adult mosquitoes were sampled using both US Centers for Disease Control miniature light traps (Model 512; John W. Hock Company, Gainesville, Florida, USA) and pyrethrum spray catches (PSC) (Fig. 2) for two consecutive days.

Per sampling round, upon receiving consent from the household heads (Fig. 3), mosquitoes were caught, in fifteen randomly selected houses, out of which ten were used for mosquito collection with CDC light traps, and the remaining five for PSC.

Fig. 2. Collection methods of adult mosquitoes using CDC light trap (a) and pyrethrum spray catch (PSC) (b) methods.

Fig. 3. Household visit and consent request.

CDC light traps were hung approximately 1.5m from the ground at the foot of a bed with one family member sleeping under an untreated bed net. Each sampling day, traps were operated overnight, from 6 pm to 6 am. In the morning, PSC started at 7 am. White sheets were laid on the floor of the surveyed houses. Thereafter, the houses were sprayed, with an aerosol (Rambo®) containing 0.25% transfluthrin and 0.20% permethrin.

All mosquitoes that fell on the white sheets were collected using forceps 15 minutes post spraying.

Mosquito processing

Mosquitoes sampled from households were taken to the Entomology Unit of National Public Health Institute of Djibouti for processing after suffocation with chloroform vapour. Female mosquitoes were kept aside, counted and morphologically identified to species by an expert entomologist using appropriate taxonomic keys by Gillies & Coetzee (1987) and Glick (1992).

Insecticide resistance testing

Collection and rearing of mosquito larvae

Between December 2016 and January 2017, field visits were carried out in the garden of Ambouli, Djibouti-ville, in search of *Anopheles* larval breeding sites. Larvae and pupae were sampled from the surface of the water using the dipping method (Fig. 4) and transported to the insectary for rearing (Fig. 5). Larvae were reared at ambient temperature and fed daily on finely ground fish food (TetraMin Tropical Flakes; Spectrum Brands, Inc. Madison, WI, USA). After emergence, the morphological identification of adult mosquitoes was performed to species-level, and only *A. stephensi* individuals were tested for insecticide resistance status. . Adult mosquitoes were kept in standard cubical cages with 30 x 30 x 30 cm iron frames covered with white netting. The cages were placed in the adult insectary at 27°C±2°C and 65–70% Relative Humidity. A photoperiod of 12 h light–12 h dark covered the night and daylight times for adult mosquitoes. A cotton wool swab soaked with 10% glucose solution was placed in the cages to feed adult mosquitoes and prevent starvation.

Fig. 4. Larval collections from *A. stephensi* breeding sites.

Fig. 5. Mosquito rearing for WHO susceptibility tube test.

Susceptibility testing

WHO susceptibility tube tests were performed by exposing emerged *A. stephensi* to nine insecticides belonging to the four insecticide classes: three pyrethroids (0.05% deltamethrin, 0.75% permethrin and 0.05% lambdacyhalothrin), three organophosphates (5% malathion, 1% fenithrotion and 0.25% pirimiphos methyl), one organochlorine (DDT 4%) and two carbamates (0.1% bendiocarb and 0.1% propoxur) (WHO, 2016). The impregnated papers were procured from Vector Control Research Unit, University Sains Malaysia, Penang, Malaysia (WHO, 2016). Approximately 100 unfed female mosquitoes aged 3-5 days were exposed to insecticides in batches of 20-25 specimens. Batches of mosquitoes exposed to untreated papers served as control. After the 1-hour exposure time, knock-down and delayed mortality were recorded respectively 60 minutes and 24 hours post-exposure. Bioassays were performed at a temperature of 27 °C ± 2 °C and a relative humidity of 75% ± 10%.

Data analysis

Data generated were initially recorded on standardised data record forms before entry into predesigned databases. Composition of mosquito populations was studied in terms of species diversity expressed as richness (Taxa S), and species abundance expressed as evenness (Simpson, 2019).

In each surveillance site, the Shannon-Wiener diversity index (H) and Simpson's dominance indexes (EH) were determined:

- $H = -\sum_i (p_i \times \ln(p_i))$ where:
  - Σ: A Greek symbol that means "sum"
  - ln: Natural log
  - pi: The proportion of the entire community made up of species i
- EH = H / ln(S) where:
  - H: The Shannon Diversity Index
  - S: The total number of unique species

Mosquito diversity was compared between sites using Student's t test under PAST 4.10.

Resistance status of wild vector populations of *A. stephensi* was determined from the percentage mortality, following WHO guidelines on insecticides susceptibility: mortality ≥ 98% indicates susceptibility; mortality less than 90% indicates resistance, while 90% to 97% mortality means a possible resistance that needs to be confirmed (WHO, 2016). Confidence intervals of mortality rates were determined using the exact binomial test on Stata version 15.0 (Stata Corp., College Station, TX).

Ethical considerations

Approval for this study was granted by the institutional ethics review board of the National Public Health Institute of Djibouti. Consents were sought from heads of households in houses where mosquitoes were collected. Authorization was requested before access to the garden of Ambouli.

Results

Mosquito species composition

Overall 12,538 host-seeking mosquitoes were collected in the study area using CDC light trap and PSC methods. The mosquito samples belonged to four genera (*Anopheles, Culex, Aedes, Uranotaenia*) and 12 species (Table II). Only 44 mosquitoes were of *Anopheles* species representing < 1% of the total number of collected samples. *An. gambiae* S.L and *A. stephensi* were the two major malaria vectors identified.

Both species were found in Ali-Sabieh, Dikhil and Tadjourah regions, while only *A. stephensi* were collected in Djibouti-ville, Obock and Arta. Secondary malaria vectors such as *A. nili somalicus, A. dthali* and *A. azaniae* were also recorded.

Table II. Mosquito species diversity and dominance in the sentinel sites over the 6 regions of Djibouti Republic.

	Djibouti-ville		Arta		Ali-Sabieh		Dikhil		Tadjourah		Obock		Total	
	ni	Pi	ni	Pi	ni	Pi	ni	Pi	ni	Pi	ni	Pi	ni	Pi
Anopheles gambiae	0	0	0	0	2	0.0008	1	0.0013	3	0.0043	0	0	6	0.0005
Anopheles stephensi	5	0.0009	3	0.0027	2	0.0008	4	0.0052	1	0.0014	1	0.0007	16	0.0013
Anopheles dthali	1	0.0002	0	0	0	0	3	0.0039	0	0	1	0.0007	5	0.0004
Anopheles nili somaliculs	0	0	0	0	0	0	0	0	2	0.0029	1	0.0007	3	0.0002
Anopheles azaniae	0	0	0	0	4	0.0015	6	0.0078	1	0.0014	3	0.0020	14	0.0011
Aedes aegypti	26	0.0045	1	0.0009	26	0.0099	12	0.0156	35	0.0503	2	0.0013	403	0.0321
Culex quinquefasciatus	5620	0.9665	1118	0.9920	2523	0.9648	729	0.9455	645	0.9267	1489	0.9848	11827	0.9434
Culex decens	125	0.0215	0	0	26	0.0099	16	0.0208	0	0	15	0.0099	182	0.0145
Culex nebulosus	12	0.0021	2	0.0018	8	0.0031	0	0	0	0	0	0	22	0.0018
Culex pupiens	11	0.0019	3	0.0027	20	0.0076	0	0	1	0.0014	0	0	37	0.0030
Culex fatigans	12	0.0021	0	0	4	0.0015	0	0	0	0	0	0	16	0.0013
Uranotaenia bilineata	3	0.0005	0	0	0	0	0	0	8	0.0115	0	0	11	0.0009
Total	5815	1	1127	1	2615	1	771	1	696	1	1512	1	12536	1
Taxas_S	9		5		9		7		8		7		12	
Shannon diversity index	0.1885		0.0570		0.2120		0.2935		0.3407		0.2668		0.2948	
Simpson's dominance index	0.0654		0.0159		0.0689		0.1052		0.1385		0.0301		0.1087	

ni= number of species i; Pi= fraction of the entire population made up of species i.

Culex quinquefasciatus was the most important mosquito species identified in the sampling locations representing 93%-99% of the collection in each of the 6 regions. Four other species of *Culex* mosquitoes (*Culex descens, Culex nebulosus, Culex pupiens*) were found at low frequency (< 3%) in all sites. *Aedes aegypti* mosquitoes were also found in the surveyed sentinel sites, mainly in the region of Tadjourah where it

represented 5% of the collection, against <2% in each of the regions of Djibouti-ville, Arta, Dikhil, Ali-Sabieh and Obock. Very few samples of *Uranotaenia bilineata* adult mosquitoes were also recorded.

Shannon-Wiener diversity index and Simpson's dominance value were lower in Arta region (0.0570 and 0.0159 respectively) than Djibouti-ville (0.1885 and 0.654), Ali-Sabieh (0.2120 and 0.0689), Dikhil (0.2935 and 0.1052), Tadjourah (0.3407 and 0.1385) and Obock (0.2668 and 0.0301). This illustrated a very little diversity in mosquito populations in all regions, particularly in Arta where only 5 mosquito species were sampled at a low frequency over the 3 collection rounds. The situation was quite similar in Djibouti-ville, Ali-Sabieh, Dikhil, Tadjourah and Obock as the number of mosquito species found per region ranged between 7 and 12.

Insecticide resistance results with wild An. stephensi

The knock-down and delayed mortality rates of the wild *A. stephensi* populations emerged from larvae collected in Djibouti-ville and exposed to discriminating doses of insecticides through WHO susceptibility tube test are presented in Figs 6 and 7, respectively.

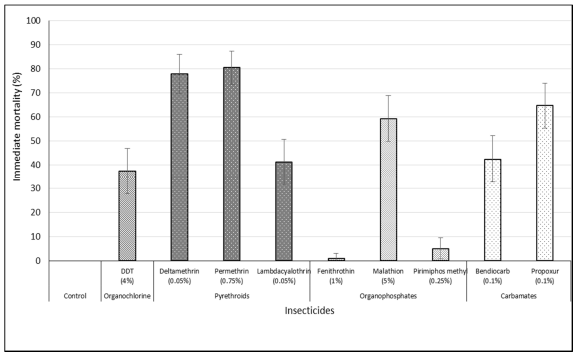

Fig. 6. Knock-down 60 minutes post-exposure in WHO tube tests with wild *Anopheles stephensi* exposed to 4 classes of insecticides. Error bars represent 95% confidence intervals.

Overall 1014 specimens of *A. stephensi* two to five days old were tested against nine different insecticides. As expected from pyrethroids, a knock-down effect on the population of *A. stephensi* was observed with deltamethrin (78%; 95% CI: 70-86%) and permethrin (80%; 95% CI: 74–87%) (Fig. 6).

The tested *A. stephensi* population of Djibouti-ville also showed a reduced knock-down effect with non-pyrethroid insecticides like the organophosphate malathion (59%; 95% CI: 50–69%) and carbamate propoxur (65%; 95% CI: 55–74%) (Fig. 5).

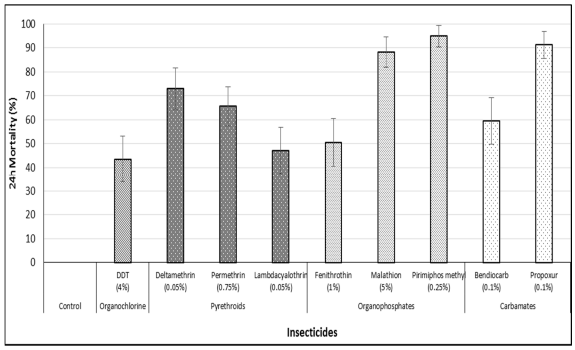

Fig. 7. 24h mortality in WHO tube tests with wild *Anopheles stephensi* exposed to 4 classes of insecticides. Error bars represent 95% confidence intervals.

Delayed mortality (24 h) in the control was very low (<5%) validating the test conditions. *A. stephensi* Djibouti-ville exhibited resistance to the organochlorine DDT with mortality of 40% (Fig. 7). Resistance to pyrethroids was also observed as the mortality with deltamethrin, permethrin and lambdacyalothrin was 73% (95% CI: 64–82%), 66% (95% CI: 53–74%) and 47% (95% CI: 37–47%) respectively (Fig. 7) indicating a high level of resistance to pyrethroids. The situation was similar with the organophosphates fenithrothion (51%; 95% CI: 41–61%) and malathion (88%; 95% CI: 82–94%) while resistance to pirimiphos-methyl (95%; 95% CI: 91–99%)and propoxur (91%; 95% CI: 86–97%) are to confirm. Resistance to the carbamates bendiocarb was observed with a lower mortality of 60% (95% CI: 51–69%).

Resistance intensity in performed bioassays

The delayed mortality of *A. stephensi* from Djibouti-ville that were exposed to 1×, 5× and 10× of the diagnostic doses of deltamethrin was 73% (95% CI: 65–81%), 88% (95% CI: 82–94%), and 96% (95% CI: 93–99%) respectively whilst for permethrin the induced mortality was 66% (95% CI: 56–74%), 99% (95% CI: 98–100%) and 100%, indicating a low to moderate resistance intensity with pyrethroids (Fig. 8). Similarly, the organophosphate pirimiphos

methyl, showed a lower resistance intensity with increasing mortality at 1×, 5× and 10× of the diagnostic doses ranging from 90% (95% CI: 85-95%) to 100% (Fig. 8).

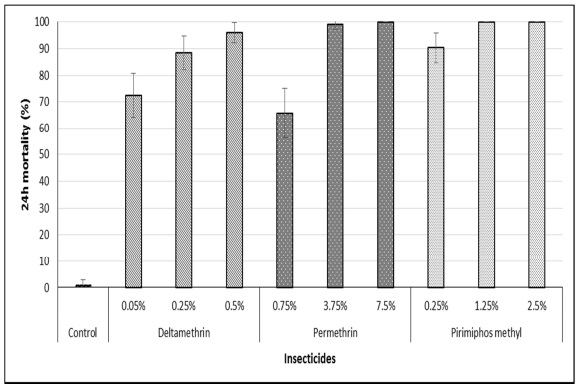

Fig. 8. Resistance intensity results with wild *Anopheles stephensi* in WHO tube test. Error bars represent 95% confidence intervals.

PBO synergist bioassays

Mortality in WHO susceptibility tube test with PBO (*Piperonyl butoxide*), deltamethrin and permethrin each alone was respectively 4% (95% CI: 0-8%), 73% (95% CI: 65-81%) and 65% (95% CI: 57-73%) in An. stephensi population from Djibouti-ville (Fig. 9). Pre-exposure to PBO resulted in a significant increase in mortality, 100% ($p<0.05$) and 93% (95% CI: 86-98%, $p<0.05$) respectively with deltamethrin and permethrin (Fig. 9), suggesting at least some involvement of P450 detoxification enzymes in pyrethroid resistance in the *A. stephensi* populations from Djibouti-ville.

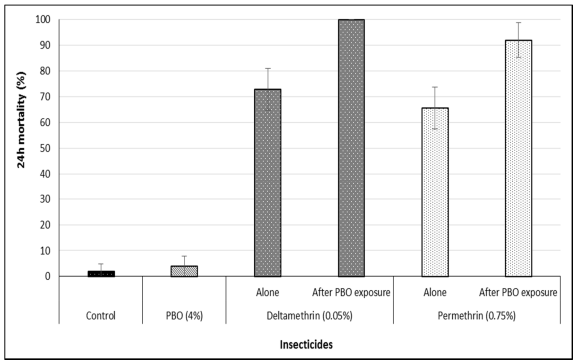

Fig. 9. Mortality (%) of wild *A. stephensi* from Djibouti-ville in WHO tube bioassays without and with pre-exposure to the synergist PBO. Error bars represent 95% confidence intervals.

Discussion

The history of repeated malaria epidemics in the Republic of Djibouti reflects flaws in malaria surveillance within the country. In fact, there has been very little or no regularity in the implementation of a rigorous entomological and epidemiological surveillance plan for malaria control at country level. Our study aimed at filling this gap by updating existing data on malaria vectors. While only morphological identification was performed by an expert taxonomist, our investigation demonstrated the presence of *A. stephensi* in all the 6 regions of Djibouti suggesting that this population have become established in the country after its first detection in Djibouti-ville in 2013 (Faulde *et al.,* 2014). The absence of molecular data for confirmation of *An. stephensi* as the major malaria vector remains a limitation. However, to minimize the risk of misidentification of *A. stephensi* specimens, we compared them with the voucher specimens caught in 2013 by Faulde *et al.* (2014). Further research studies have finally confirmed our observation and reported a key role of *A. stephensi* in the occurrence of another outbreak of *P. falciparum* and *P. vivax* malaria in Djibouti in 2019 (Seyfarth *et al.*, 2019; De Santi *et al.,* 2019). This species was reported to invade urban locations in neighbouring Ethiopia (Takken & Lindsay, 2019), Somalia (Ali *et al.,* 2022), Sudan (Ahmed *et al.,* 2021), and appeared to be spreading to other areas of Africa as recently identified in Nigeria, West Africa. With more than 40% of the population in Africa living in urban areas , the invasion and spread of *A. stephensi* calls for urgent actions to prevent urban malaria epidemics threatening efforts for malaria control and elimination in Africa (WHO, 2022). It is an alarm call to local health authorities, particularly the NMCP for appropriate entomological surveillance measures and effective control tools against this new malaria vector in the country.

The recent invasion of the cities of endemic countries by *A. stephensi,* a vector originally found in South Asia and the Middle East, including large parts of the Arabian Peninsula, should not overshadow the threat posed by other dreadful vector species such as those of the *A. gambiae* S.L. and *A. funestus* GR. which

continue to play a predominant role in malaria transmission in Sub-Saharan Africa settings (Ngongang-Yipmo *et al.,* 2022; Oliver *et al.,* 2022, Nkemngo *et al.*, 2020; Pinda *et al.,* 2022). In the context of Djibouti where we identified the presence of *A. gambiae* SL. alongside with the newly established *A. stephensi* species, it would be crucial to design and implement further integrated vector control strategies which take into account bionomics and specificity of both species to consider the challenges of their cohabitation.

The *A. stephensi* population of Djibouti-ville demonstrated resistance to pyrethroids with a low to moderate intensity and involvement of P450 detoxification enzymes. The phenotypic insecticide resistance to pyrethroids in *Anopheles* vectors is widespread in all regions of Africa (Sanou *et al.,* 2021; Hemingway & Ranson, 2000, Ranson & Lissenden, 2016) including *A. stephensi* (Enayati *et al.,* 2020) and outside Africa (Yared *et al.,* 2020; Safi *et al.,* 2017). Pre-exposure to the Synergist PBO led to a restoration of susceptibility for *A. stephensi* from Djibouti-ville revealing the role of detoxifying metabolic enzymes in the insecticide resistance in this population. Therefore deployment of PBO nets may provide a greater efficacy in the control of *A. stephensi* in urban Djibouti as previously reported in many settings with pyrethroid-resistant malaria mosquitoes across Africa (Corbel *et al.*, 2010).

Phenotypic resistance to organophosphate and carbamate was also observed in *A. stephensi*. However it would be interesting to investigate mechanisms conferring insecticide resistance (Singh *et al.,* 2011; Gorouhi *et al.,* 2016) in order to implement an appropriate management of multi-resistance populations *A. stephensi* in Djibouti and support choices of vector control interventions (WHO, 2012; Ghosh, 2020). The presence of *A. stephensi* in all the 6 regions of the Republic of Djibouti and the neighbouring Ethiopia, Eritrea, Somalia recommends to set up a national network for a continuous monitoring of insecticide resistance to regularly update insecticide resistance status and support decision-making.

Conclusion

In this study we identified *A. stephensi* and *A. gambiae* SL. as the two major malaria vectors in the Republic of Djibouti. *A. stephensi* mosquitoes was present in all the 6 regions investigated suggesting that this species has become established in the country after its first detection in only Djibouti-ville in 2013. *A. nili somalicus, A. dthali* and *A. azaniae* were the secondary malaria vectors collected across the South-North transect. Insecticide resistance testing of the *A. stephensi* populations from Djibouti showed phenotypic resistance to organochlorine, pyrethroids, organophosphates and carbamates revealing the urgency to develop and implement a programme for monitoring and managing insecticide resistance in local vector populations with efficient control strategies at country level.

Competing Interests

The authors declare that they have no competing interests.

Funding

The study was part of the technical support to the Djibouti Ministry of Heath by the Global Fund to Fight AIDS, Tuberculosis and Malaria via the United Nations Development Programme. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Authors Contributions

RG wrote the main study protocol and design the study. HMO, MMI and RG supervised the study data collections. RG performed data analysis. RG wrote the initial draft of the manuscript, which was revised by AS. HYD and AOB provided administrative and logistics support. All authors read and approved the final manuscript.

Acknowledgements

We thank Angela Anna de Tommasi, Aicha Mohamed Ali and Dr. Ignace Bimenyimana of United Nations Development Programme for their support during the conduct of activities. We also thank the technical staff of Djibouti NMCP and

NPHI for their assistance. We are grateful to heads of households for their kind collaboration.

References

Ahmed A. 2021. Invasive Malaria Vector *Anopheles stephensi* Mosquitoes in Sudan, 2016-2018. Emerg Infect Dis **27(11),** 2952-2954.

Ali S. 2022. Morphological identification and genetic characterization of *Anopheles stephensi* in Somaliland. Parasit Vectors **15(1),** 247.

Carteron B, Morvan D, Rodhain F. 1979. The control of culicidae in Djibouti town. An experience of 7 years (Author's transl)]. Med Trop **39(5),** 555-8.

Corbel V, Chabi J, Dabiré RK, Etang J, Nwane P, Pigeon O, Hougard JM. 2010. Field efficacy of a new mosaic long-lasting mosquito net (PermaNet® 3.0) against pyrethroid-resistant malaria vectors: A multi centre study in Western and Central Africa. Malaria Journal **9(1),** 1-12.

Crowell VHD, Briët O, Chitnis N, Maire N, Smith T. 2012. Can we depend on case management to prevent re-establishment of *P. falciparum* malaria, after local interruption of transmission. Epidemics **4,** 1-8.

de Santi VP. 2021. Role of Anopheles stephensi Mosquitoes in Malaria Outbreak, Djibouti, 2019. Emerg Infect Dis **27(6),** 1697-1700.

Enayati A. 2020. Evolution of insecticide resistance and its mechanisms in *Anopheles stephensi* in the WHO Eastern Mediterranean Region. Malar J **19(1),** 258.

Faulde MK, Rueda LM, Khaireh BA. 2014. First record of the Asian malaria vector *Anopheles stephensi* and its possible role in the resurgence of malaria in Djibouti, Horn of Africa. Acta Trop **139,** 39-43.

Faulde MK, Spiesberger M, Abbas B. 2012. Sentinel-site-enhanced near-real time surveillance documenting West Nile virus circulation in two *Culex* mosquito species indicating different transmission characteristics, Djibouti City, Djibouti. J. Egypt. Soc. Parasitol **42,** 281-294.

Faulde MK, Ahmed AA. 2010. Haematophageous vector monitoring in Djibouti city from 2008 to 2009: first records of *Culex pipiens* ssp. torridus (IGLISCH), and *Anopheles sergentii* (Theobald). J Egypt Soc Parasitol, **40(2),** 281-94.

Fox E, Abbate EA, Leef M, Mikhail E, Said-Salah Y, Hassan A. 1989. Malaria in the Djibouti Republic: Results of a serologic survey in Ambouli. Med. Trop **51,** 185-189.

Fox E, Bouloumie J, Olson JG, Tible D, Lluberas M, Shakib SO, Parra JP, Rodier G. 1991. *Plasmodium falciparum* voyage en train d'Éthiopie à Djibouti. Med. Trop **51,** 185-189.

Ghosh SK. 2020. *Anopheles* (Cellia) stephensi Liston 1901: the vector of urban malaria- An imminent threat to malaria elimination in India. In: Vector Biology and Control, an update for malaria elimination initiative in India (Dev V, Editor), The National Academy of Sciences, India pp. 69-82.

Gorouhi MA. 2016. Current Susceptibility Status of *Anopheles stephensi* (Diptera: Culicidae) to Different Imagicides in a Malarious Area, Southeastern of Iran. J. Arthropod Borne Dis **10(4),** 493-500.

Gillies MT, Coetzee M. 1987. A supplement to the Anophelinae of Africa South of the Sahara. Publ S Afr Inst Med Res **55,** 1-143.

Glick JI. 1992. Illustrated key to the female *Anopheles* of southwestern Asia and Egypt (Diptera: Culicidae). Mosq Syst **24,** 125-153.

Hatemmm. 1996. Health development in Djibouti. World Health Forum **17(4),** 390-1.

Hemingway J, Ranson H. 2020. Insecticide resistance in insect vectors of human disease. Annual review of entomology **45(1),** 371-391.

Khaireh BA. 2013. Population genetics analysis during the elimination process of *Plasmodium falciparum* in Djibouti. Malar J **12,** 201.

Louis JP. 1988. Malaria in the Republic of Djibouti. Strategy for control using a biological antilarval campaign: Indigenous larvivorous fishes (*Aphanius dispar*) and bacterial toxins. Med Trop **48,** 127-131.

Ngongang-Yipmo ES. 2022. Reduced performance of community bednets against pyrethroid-resistant *Anopheles funestus* and *Anopheles gambiae*, major malaria vectors in Cameroon. Parasit Vectors **15(1),** 230.

Nkemngo FN. 2020. Multiple insecticide resistance and *Plasmodium infection* in the principal malaria vectors *Anopheles funestus* and *Anopheles gambiae* in a forested locality close to the Yaounde airport, Cameroon. Wellcome Open Res **5,** 146.

Oliver SV, Lyons CL, Brooke BD. 2022. The effect of blood feeding on insecticide resistance intensity and adult longevity in the major malaria vector *Anopheles funestus* (Diptera: Culicidae). Sci Rep **12(1),** 3877.

Pinda PG. 2020. Comparative assessment of insecticide resistance phenotypes in two major malaria vectors, *Anopheles funestus* and *Anopheles arabiensis* in south-eastern Tanzania. Malar J **19(1),** 408.

Ranson H, Lissenden N. 2016. Insecticide resistance in African *Anopheles* mosquitoes: A worsening situation that needs urgent action to maintain malaria control. Trends in Parasitology **32(2),** 187-196.

Rodier GR. 1995. Recurrence and emergence of infectious diseases in Djibouti city. Bull World Health Organ **73(6),** 755-9.

Rogier C. 2005. Malaria epidemic and drug resistance, Djibouti. Emerg Infect Dis **11(2),** 317-21.

Safi NH. 2017. Evidence of metabolic mechanisms playing a role in multiple insecticides resistance in *Anopheles stephensi* populations from Afghanistan. Malar J **16(1),** 100.

Sanou A, Nelli L, Guelbéogo WM, Cissé F, Tapsoba M, Ouédraogo P, Ferguson HM. 2021. Insecticide resistance and behavioural adaptation as a response to long-lasting insecticidal net deployment in malaria vectors in the Cascades region of Burkina Faso. Scientific reports **11(1),** 1-14.

Schulman S. 2019. Climate Change Challenges and Djibouti. The RUSI Journal **164(1),** p. 62-75.

Seyfarth M. 2019. Five years following first detection of *Anopheles stephensi* (Diptera: Culicidae) in Djibouti, Horn of Africa: populations established malaria emerging. Parasitol Res **118(3),** 725-732.

Simpson EH. 1949. Measurement of Diversity. Nature **163,** 688-688.

Singh OPDC, Lather M, Agrawal OP, Adak T. 2011. Knockdown resistance (kdr)-like mutations in the voltage-gated sodium channel of a malaria vector *Anopheles stephensi* and PCR assays for their detection. Malar Journal **10(59).**

Takken W, Lindsay S. 2019. Increased Threat of Urban Malaria from *Anopheles stephensi* Mosquitoes, Africa. Emerg Infect Dis **25(7),** 1431-1433.

United Nations Developement Programme. 2013. Djibouti: Appel Global–Revue à mi-Parcours.

WHO. 2012. World malaria report 2011. 2012.

WHO. 2016. Test procedures for insecticide resistance monitoring in malaria vector mosquitoes.

WHO. 2022. WHO initiative to stop the spread of *Anopheles* stephensi in Africa.

World Health Organization. 2012. Global plan for insecticide resistance management in malaria vectors. World Health Organization.

Yared S. 2020. Insecticide resistance in *Anopheles stephensi* in Somali Region, eastern Ethiopia. Malar J **19(1),** 180.