Saving Lives Through
Quality Research

Phenotypic and molecular characterization of pyrethroid resistance escalation in the African malaria vector *Anopheles funestus* Cameroon-wide

Hervé Raoul TAZOKONG, Magellan Tchouakui, Gadji Mahamat, Stevia Ntadoun Tchamga, Tatiane Assatse, Steve Valdi Djova, Benjamin D. Menze, Ebai Terence, Micareme Tchoupo, Leon M. J. Mugenzi, Onana Boyomo, Charles S. Wondji

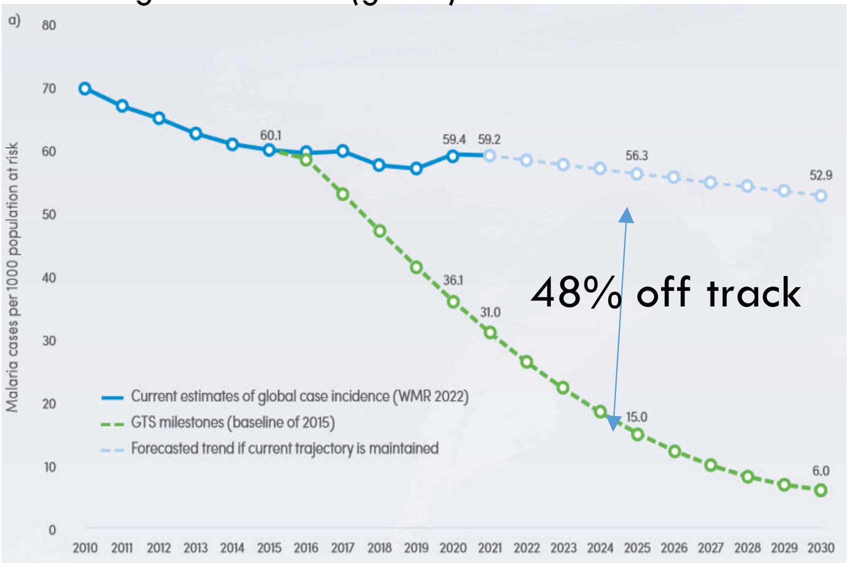
19th Annual Meeting RBM Vector Control Working Group
April 15-17, 2024, Radisson Blu Hotel, Kigali, Rwanda

Outline

- Problem Statement
- Rationale
- Methodology
- Results
- Take home message

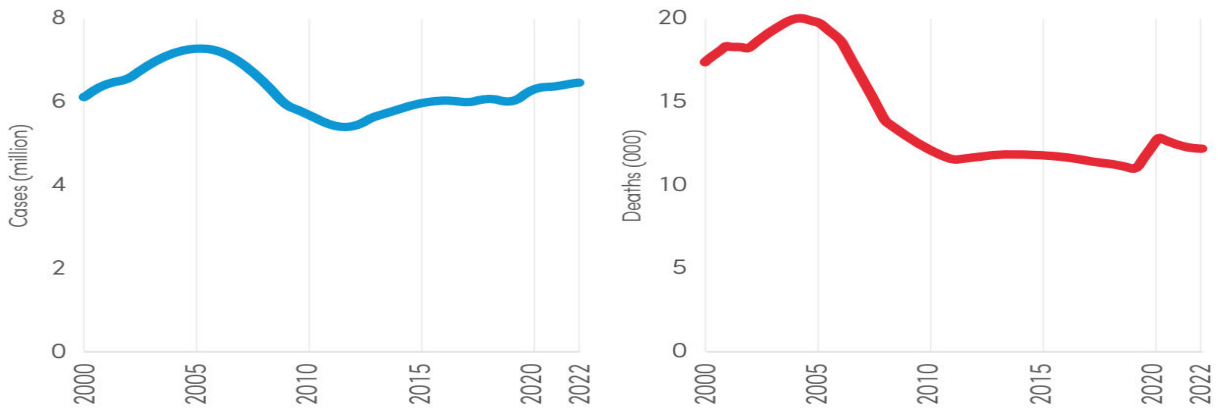
Problem Statement

Global progress in malaria case incidence considering two scenarios: current trajectory maintained (blue) and GTS targets achieved (green) Source: WHO estimates

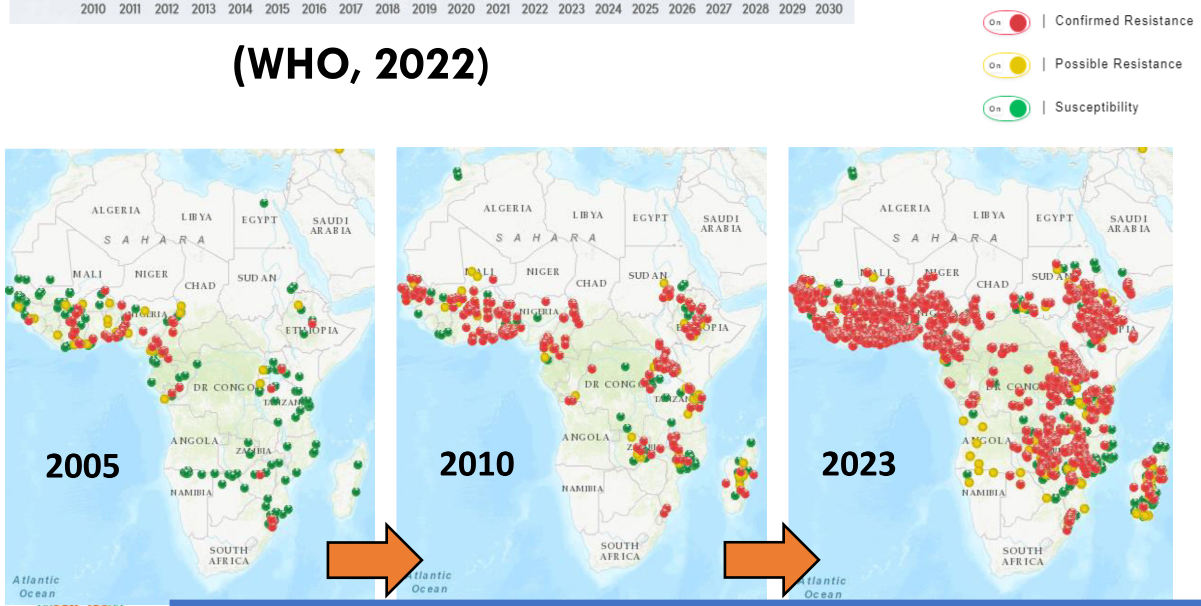


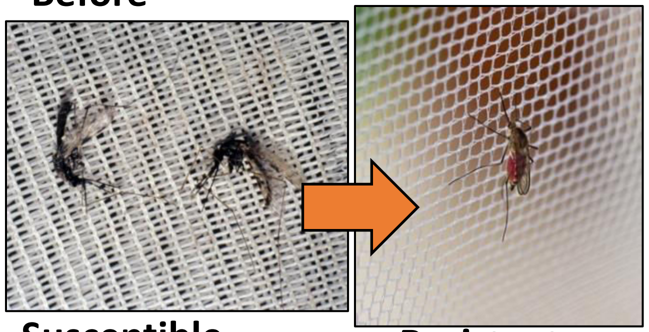
(WHO, 2022)

"Pyrethroid resistance" a challenge to malaria control



Malaria burden in Cameroon 2000-2022
WHO, 2023



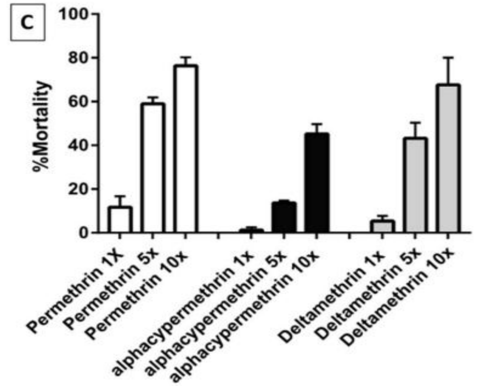


- Progress is plateauing since 2015
  - *P. falciparum* escaping detection by RDTs
  - Drug and **Insecticide resistance**
  - Climate change
  - Disruption of services due to COVID-19

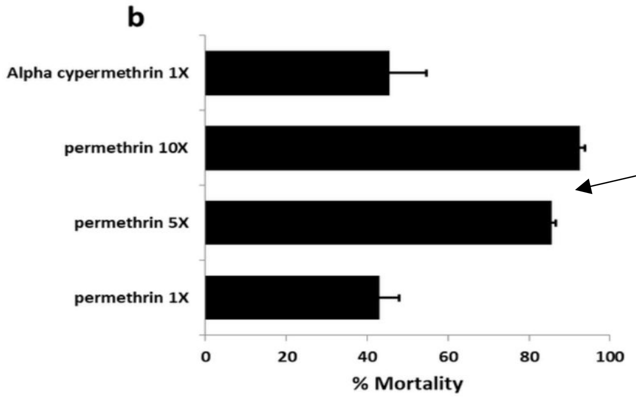
WHO, 2023

Rationale

Intensification of insecticide resistance in *An. funestus* is widespread across Africa

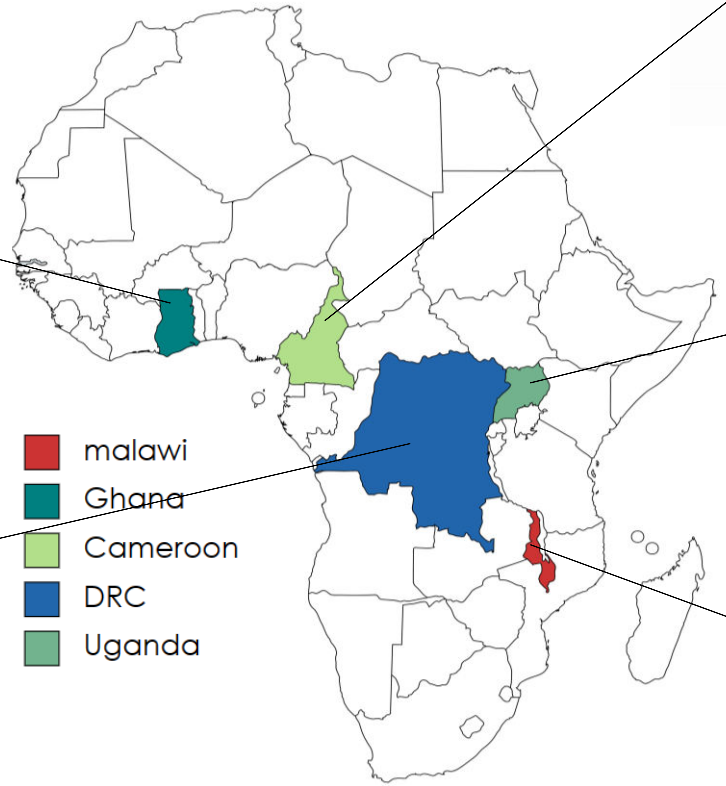


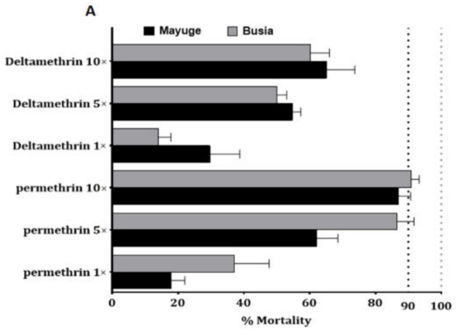
Ghana (Mugenzi et al. 2022)



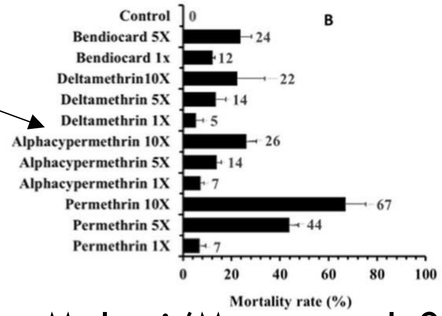
DRC (Nguiffo-Nguette et al. 2023)

Cameroon





Uganda (Tchouakui et al. 2021)



Malawi (Menze et al. 2022)

Characterizing the intensity of insecticide resistance is crucial for better resistance management strategies and before it is potentially too late, to mitigate its public health impact.

General Objective

Characterize the level of resistance, assess impact on control tools and investigate its molecular mechanisms in the major malaria vector *Anopheles funestus* in Cameroon

Methodology 1/2

Period: Aug.2020-Dec. 2021

Fig1. Map of Cameroon showing sites where indoor mosquito were collected

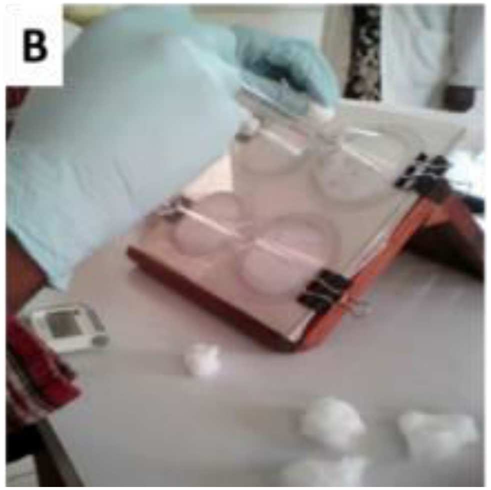
Indoor collection

Transportation to CRID lab

Rearing at the insectary

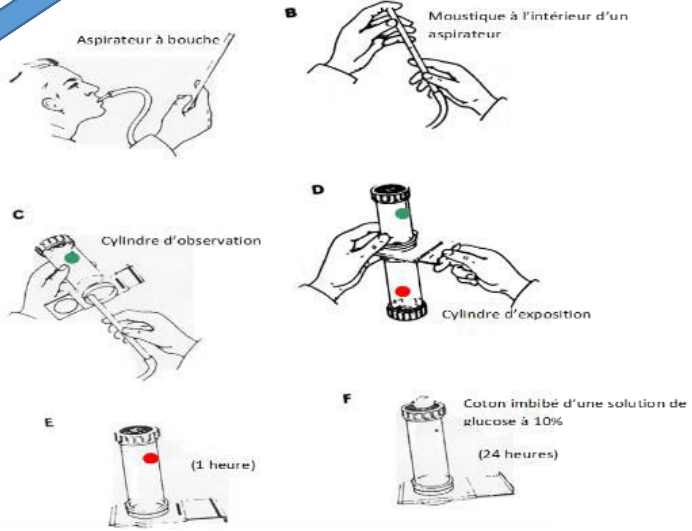
F1aged 3-5 days

Cone Assay



Various bed nets

WHO tube assay



1x, 5x and 10x DC Synergist assay

Methodology 2/2

F0 and F1 samples

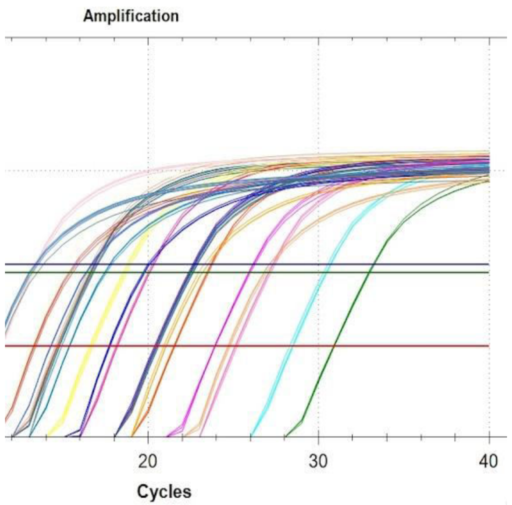
DNA extraction
Livak, 1984

DNA

+ MIX

- ID species cocktail funestus (Koekemoer *et al.* 2002)
- 6.5 Kb SV, GSTe2, CYP6P9a and b Genotyping on F0
- L119F-GSTe2 mutation on dead/alive at 1x, 5x and 10x
- Nested PCR for Plasmodium confirmation (head and thorax)

RNA extraction



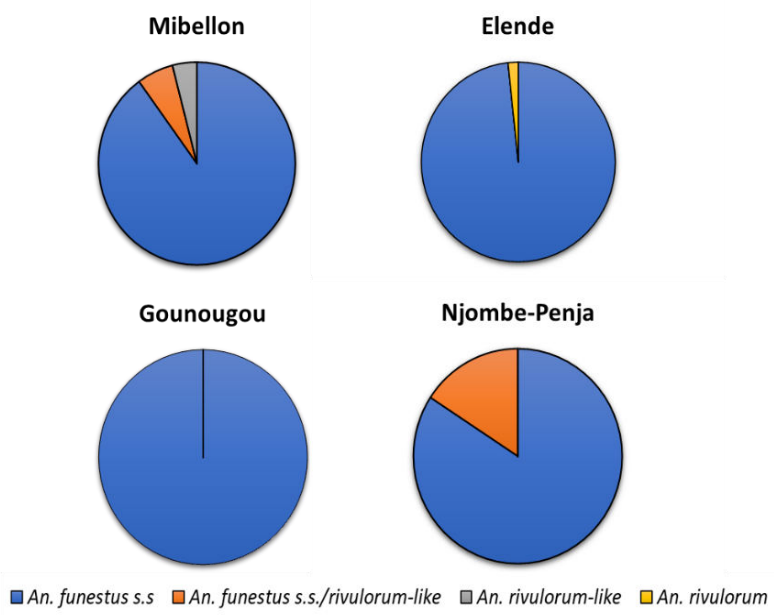
qPCR on various resistant genes including Cytochrome P450s, COE and GSTe2

Microsoft Excel 2019

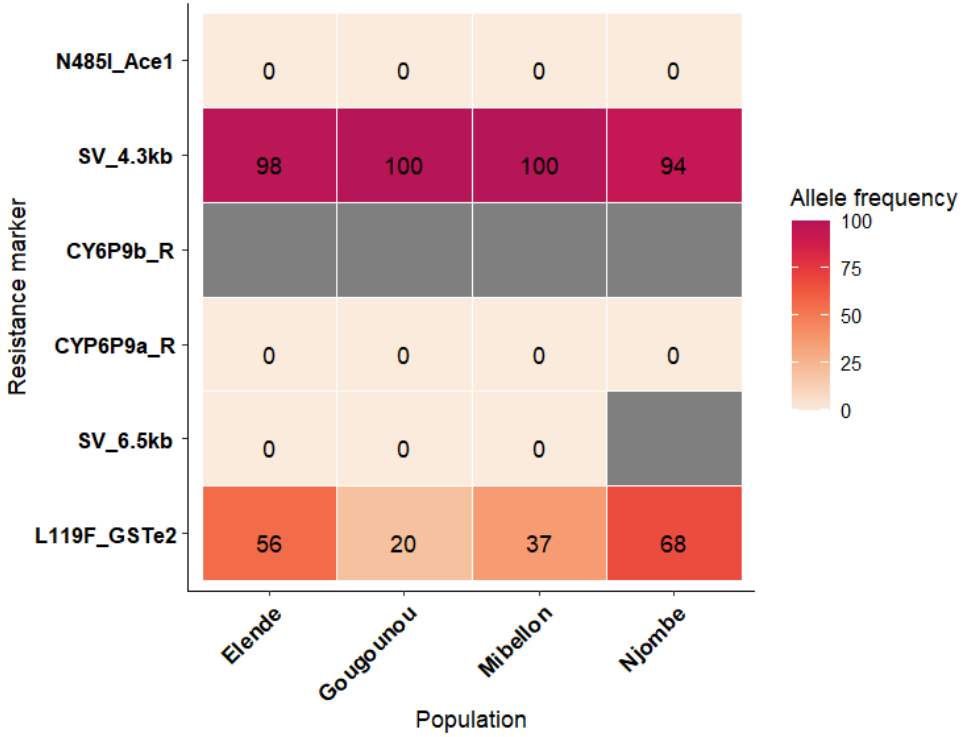
Prism 9

Graphpad

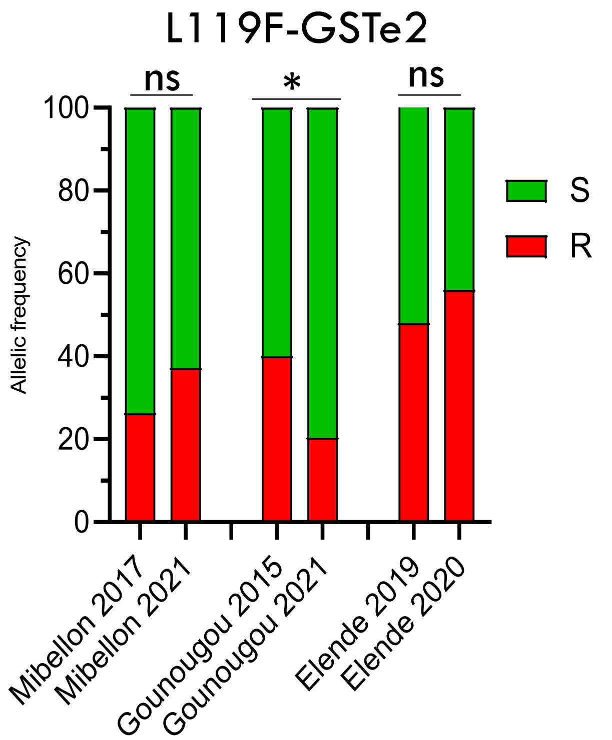
Species composition, infection rate and resistance makers distribution in field mosquitoes



Species composition in different localities



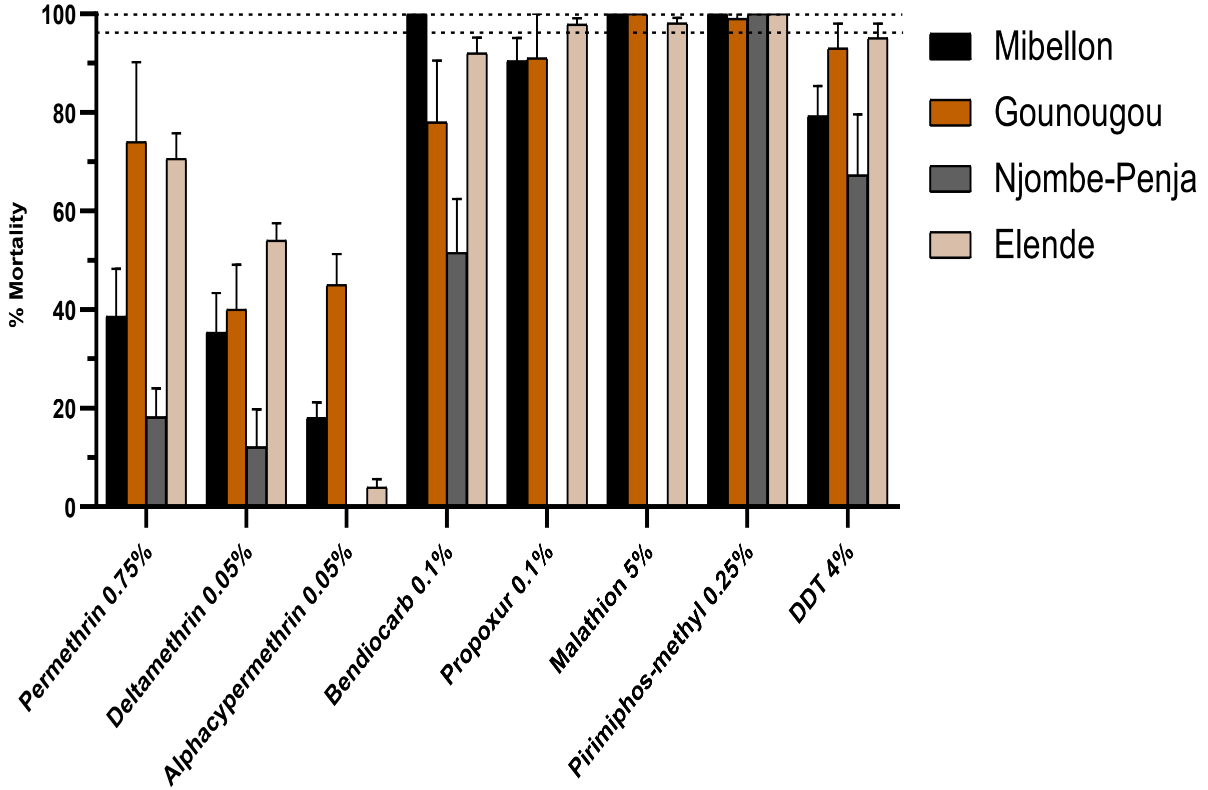
Grey indicate no amplification

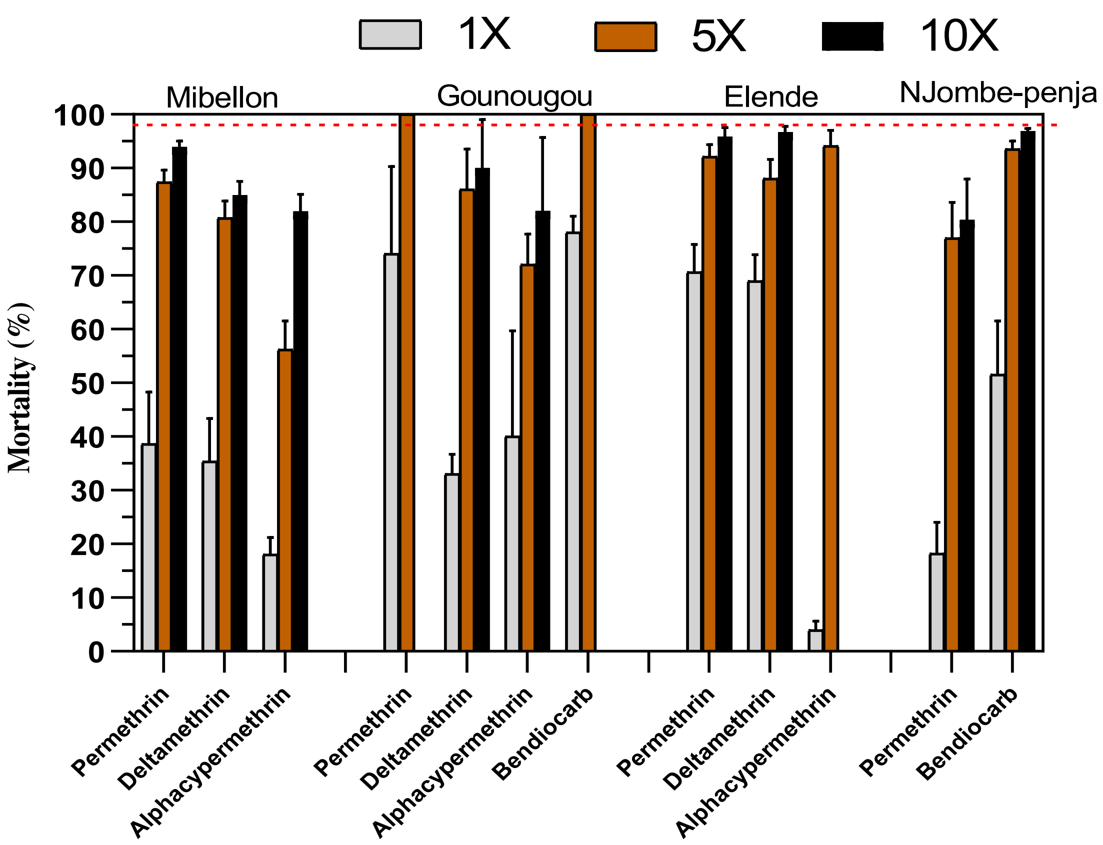


Distribution and temporal assessment of known resistant markers in F0

- *Anopheles funestus* s.s was the predominant species identified in the funestus group
- 5.4% (5/92) and 16.5% (14/85) infection rate in Mibellon and Elende respectively
- L119F-GSTe2 moderate to high frequency whereas 4.3kb SV fixed
- The 119F-GSTe2 allele increased in frequency in two sites but decreased significantly in Gounougou

Multiple and intensification of insecticide resistance in *An. funestus*





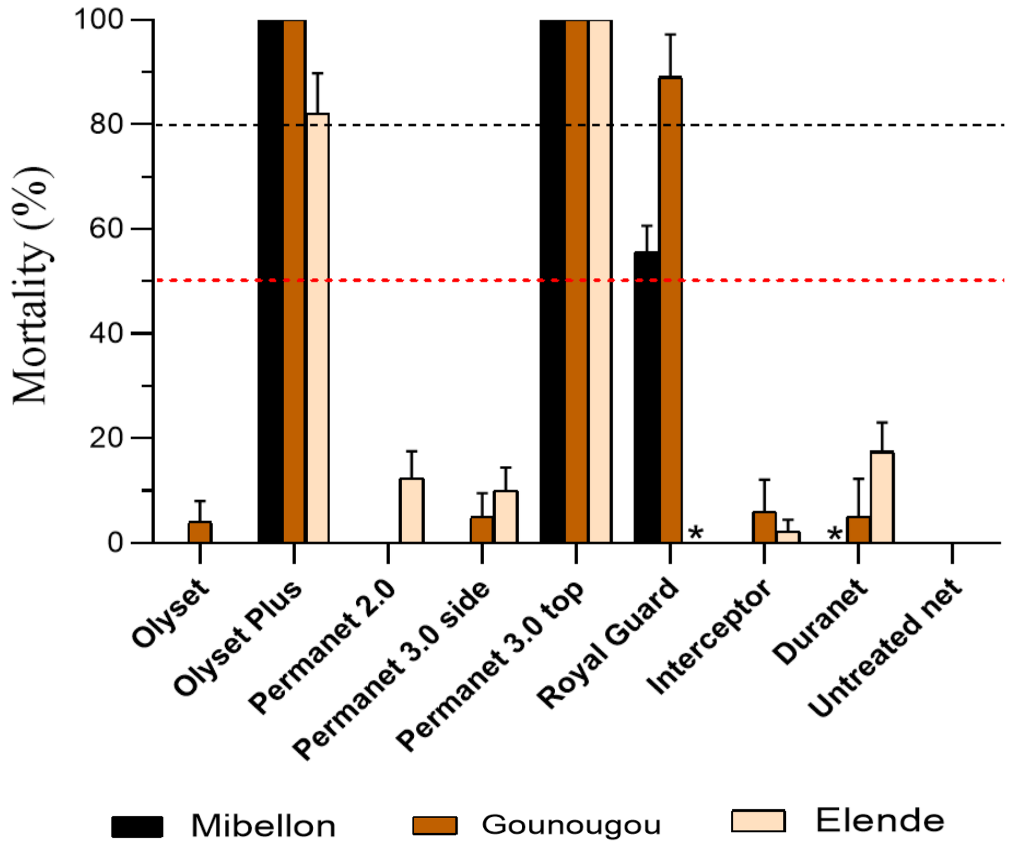
- Resistance was noticed to all the insecticide tested in at least one insecticide except for organophosphate
- High intensity of pyrethroid resistance to both type 1 and 2 was observed in all localities except in Gounougou
- High level of bendiocarb resistance was recorded in Njombe but moderate in Gounougou

Synergist assay with PBO and Bed net efficacy against *An. funestus*

Table1: synergist assay with PBO

Localities	Insecticide	Insecticide alone Mortality rate (%)	PBO + Insecticide Mortality rate (%)	P-value	95% CI
Control	None	/	0	/	/
Elende	Permethrin 1x	70.6 ± 5.21	97.1 ± 1.8	< 0.0001	16.0-37.5
	Deltamethrin 1x	54.0 ± 3.5	100.0	< 0.0001	35.9-55.9
	Alphacypermethrin 1x	16.3 ± 4.5	100.0	<0.0001	74.8-89.4
Njombe-Penja	Permethrin 1x	18.2 ± 5.8	92.9 ± 1.5		
	Bendiocarb 1x	70.8	93.7	< 0.0001	23.8-52.2
Gounougou	Permethrin 1x	74.0 ± 16.2	100.0	< 0.0001	17.8-34.6
	Deltamethrin	33.0 ± 3.7	100.0	< 0.0001	52.5-78.3
	Alphacypermethrin 1x	45.0 ± 6.3	100.0	< 0.0001	44.2-65.0
	Bendiocarb 1x	50.9 ± 12.5	93.0	< 0.0001	28.1-55.6
Mibellon	Permethrin 1x	38.6 ± 9.7	92.8 ± 7.1	< 0.0001	41.1-64.4
	Deltamethrin 1x	35.3 ± 8.4	90.6 ± 2.4	< 0.0001	43.4-64.1
	Alphacypermethrin 1x	18.1 ± 3.1	95.2 ± 4.7	< 0.0001	63.6-82.5

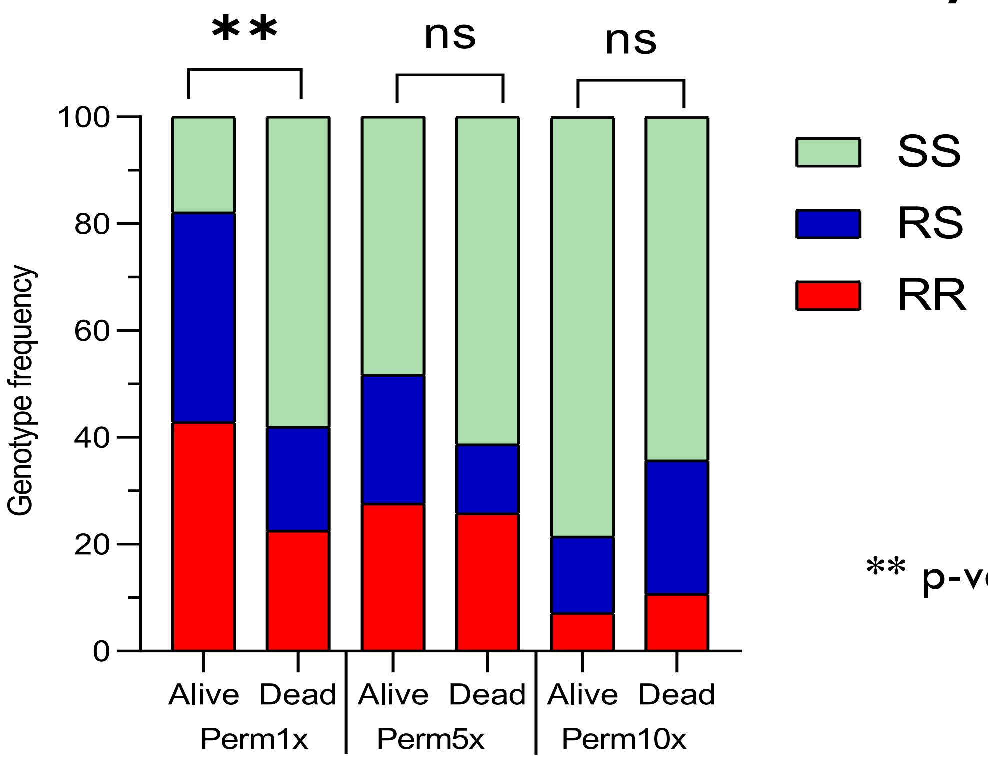
CI= Confidence Interval, PBO= Piperonyl butoxide



Efficacy of LLINs against high resistant strain of *An. funestus*

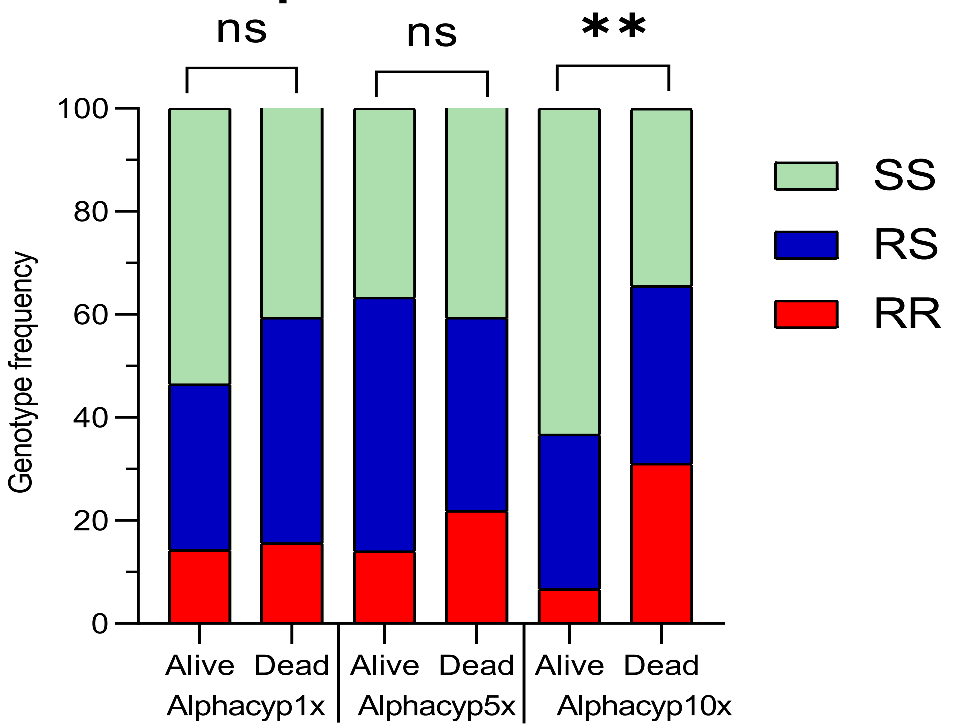
- Increased susceptibility with PBO indicating the implication of cytochrome P450s
- Drastic loss in efficacy with pyrethroids-only net but good performance with PBO nets

Association of the L119F-GSTe2 mutation and pyrethroid resistance intensity in Mibellon mosquitoes



** p-value < 0. 001

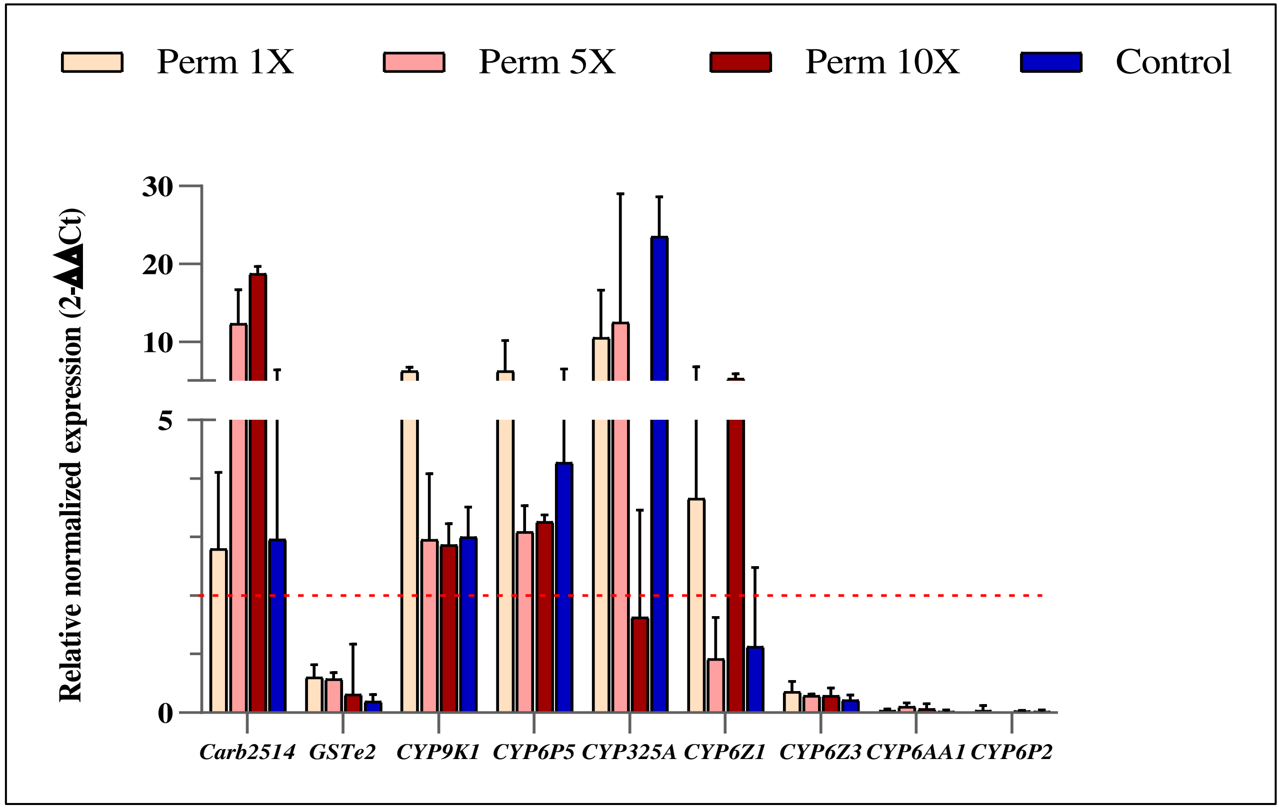
Association between GSTe2 genotype and phenotype upon permethrin exposure



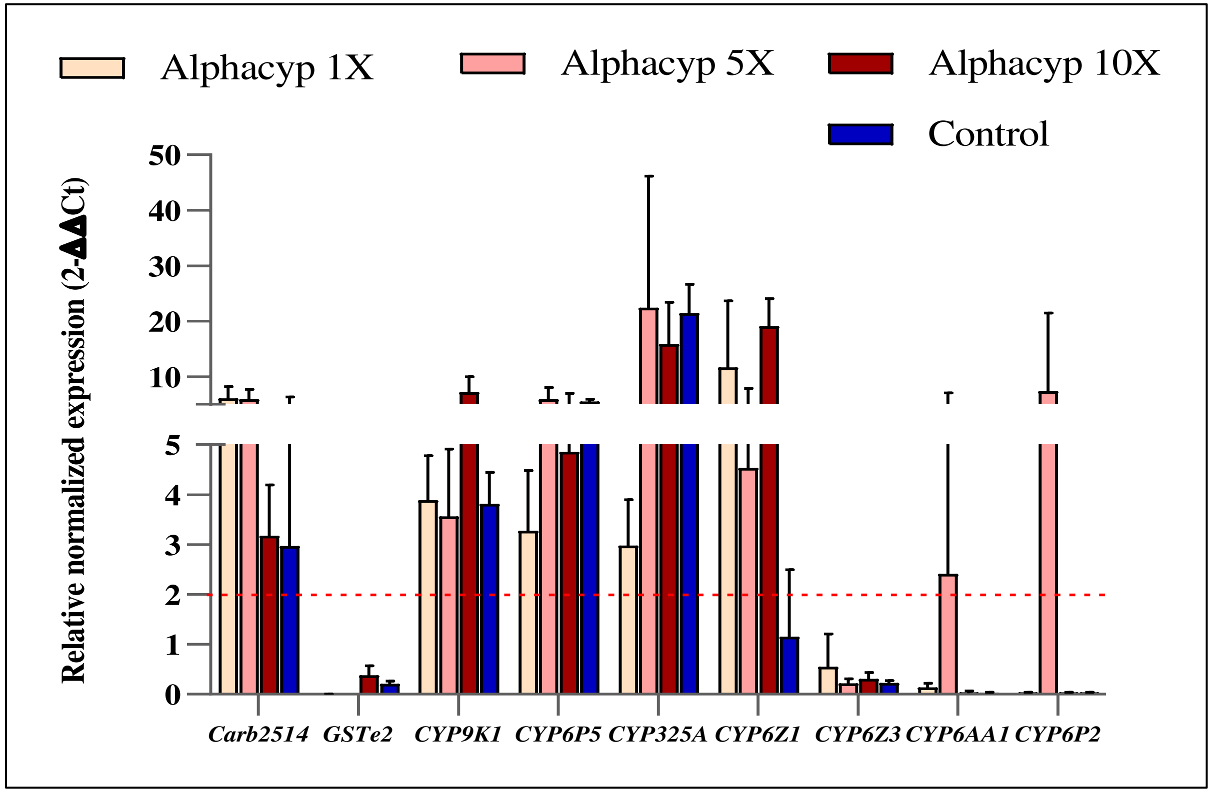
Association between GSTe2 genotype and phenotype upon a-cypermethrin exposure

- The L119F-GSTe2 mutation was associated with the ability to survive 1x DC permethrin but not 5x and 10x doses
- In contrast, a negative correlation of this mutation was noticed with a-cypermethrin 10x
- L119F-GSTe2 may impact permethrin-based nets but not the alphacypermethrin-based nets

Transcription profile of resistant genes in Mibellon population



Gene expression level in permethrin resistant mosquitoes



Gene expression level in a-cypermethrin resistant mosquitoes

- Carb2514, CYP6Z1, CYP9K1, CYP6P5 and CYP325A were upregulated in resistant mosquitoes at 1x, 5x and 10x DC
- Those genes can be used as molecular markers to monitor insecticide resistance in these locations
- No further association was noticed between the expression of those candidate genes and increasing doses of pyrethroid

Take home message

- High intensity of pyrethroid resistance recorded in all four *An. funestus* populations studied leading to a drastic loss of efficacy of pyrethroids only nets
- Aggravation of resistance may be driven by metabolic enzymes suggesting the effectiveness of PBO-based nets in these areas
- This study underscores the role of molecular surveillance of malaria vectors using existing resistant markers as key component of vector control strategies
- Whole genome sequencing techniques are needed to decipher the exact role of such resistant escalation to improve insecticides-based interventions

Manuscript: "Nationwide susceptibility profiling of *Anopheles funestus* from Cameroon reveals escalating pyrethroid resistance and reduced bed nets efficacy", in prep

Thank you for your keen Attention!

Acknowledgements

RBM Partnership To End Malaria

Schweizerische Eidgenossenschaft
Confédération suisse
Confederazione Svizzera
Confederaziun svizra
Swiss Agency for Development and Cooperation

wellcome trust
SENIOR FELOWSHIP RENEWED PROJECT Prof. WONDJI

Centre for Research in Infectious Diseases CRID

Death to Mosquitoes

Université de Yaoundé I – The University of Yaoundé I

LSTM LIVERPOOL SCHOOL OF TROPICAL MEDICINE