USAID
FROM THE AMERICAN PEOPLE

U.S. PRESIDENT'S MALARIA INITIATIVE

U.S. PRESIDENT'S MALARIA INITIATIVE
vectorlink
INTELLIGENT › INNOVATIVE › INTEGRATED

PMI VECTORLINK ADDENDUM TO MALI ENTOMOLOGICAL MONITORING ANNUAL REPORT JANUARY–DECEMBER 2019

Recommended Citation: The President's Malaria Initiative (PMI) VectorLink Project TO2. July 2020. *Addendum to Entomological Monitoring Annual Report, January–December 2019*. Rockville, MD. Abt Associates.

Contract: AID-OAA-I-17-00008

Task Order: AID-OAA-TO-17-00027

Submitted to: United States Agency for International Development/PMI

Submitted on: 8 December 2020

Approved on: 19 January 2020

TABLE OF CONTENTS

Contents

ACRONYMS

COVID 19	Corona Virus Disease 2019
CDC	Centers for Disease Control and Prevention
HLC	Human Landing Catch
Kdr	Knock down resistance
LBMA	Laboratoire de Biologie Moleculaire Appliquee
PCR	Polymerase Chain Reaction
PMI	President's Malaria Initiative
PSC	Pyrethrum Spray Catch
VGSC	Voltage Gated Sodium Channel
WHO	World Health Organization

I. INTRODUCTION

This report is an addendum to the Entomological Monitoring Annual Report, January–December 2019, submitted by the President's Malaria Initiative (PMI) VectorLink Mali project in March 2020. This addendum presents laboratory data on molecular species identification and resistance mechanisms associated with pyrethroid resistance, including voltage gated sodium channel (*Vgsc*) allele frequency for *Anopheles* mosquitoes that were collected in 2019. Specifically, it describes molecular species identification of *An. gambiae* s.l. samples that were collected monthly at the longitudinal IRS sites of Djenné, Mopti, and Bandiagara, where IRS was conducted, and in the control site of Tominian. We also determined species identification and frequency of Voltage Gated Sodium Channel *(Vgsc*-1014F (formerly kdr-west) and -1014S (formerly kdr-east) alleles (resistance mechanisms linked to pyrethroid resistance) in *An. gambiae* s.l. that were used for susceptibility tests in thirteen sentinel sites monitored in Mali. These data were not available when the annual report was submitted due to a failure of the PCR machine at the *Laboratoire de Biologie Moléculaire Appliquée* (LBMA) laboratory and coronavirus disease 2019 (COVID-19) restrictions on laboratory access from March to June 2020.

2. METHODOLOGY

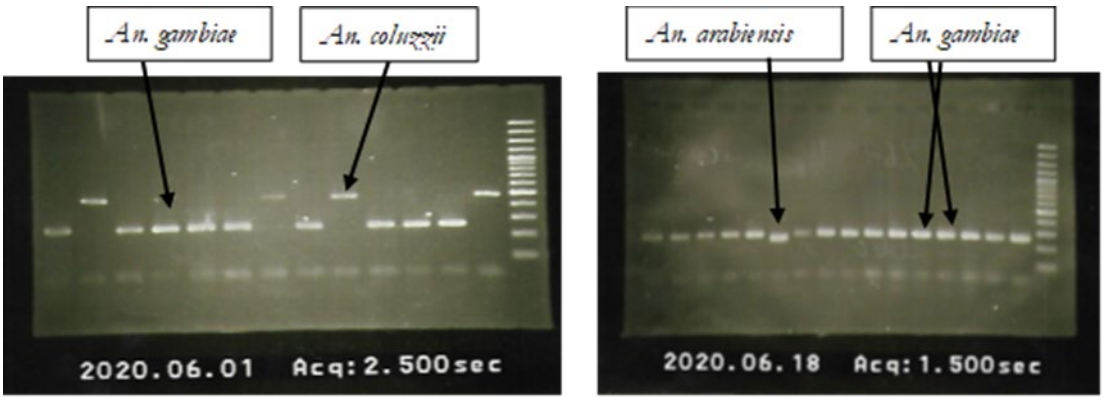
2.1 PROTOCOLS USED

All molecular analyses were conducted in the LBMA laboratory in the University of Sciences, Techniques and Technologies of Bamako (USTTB). The mosquito samples collected from sentinel sites were stored individually in Eppendorf tubes with ethanol and a subsample was sent to LBMA for molecular analysis. Technicians conducted laboratory analyses under the supervision of LBMA scientists following the protocols described below in Table 1. Molecular species identification was conducted based on the protocol of Santolamazza et al. (2008)[1] to differentiate between *An. gambiae*, *An. coluzzii,* and *An. arabiensis* (Figure 1). An adapted protocol for detection of Vgsc-1014F and1014S frequency was conducted based on the protocol of Huynh et al. (2007)[1], following optimization at LBMA.

Table 1: Protocols used for laboratory analyses of *Anopheles* malaria vectors

Molecular Analysis	Protocol	Output
PCR	Santolamazza et al. (2008)	Species identification: Identified *An. gambiae* complex sibling species including *An. coluzzii*, *An. gambiae,* and *An. arabiensis.*
	Huynh et al. (2007)	*Vgsc*-L1014F ; *Vgsc*-L1014S & Ace[1] allele frequency: Monitored pyrethroid target site resistance mechanism frequency

Figure 1: PCR gels using the protocol of Santolamazza et al. (2008) showing bands for *An. coluzzii, An. gambiae*, and *An. arabiensis* (*An. coluzzii*: 479bp, *An. gambiae* 249bp, *An. arabiensis*: 223bp).



2.2 SAMPLES TESTED

Molecular analyses were conducted on mosquitoes collected in 4 sites (3 IRS sites and 1 unsprayed site) on a monthly basis from May to December 2019. Morphological identification of *Anopheles* mosquitoes was

[1]Santolamazza F, Mancini E, Simard F, Qi Y, Tu Z, della Torre A. 2008. Structure and insertion polymorphisms of SINE 200 retrotransposons in genomic islands of speciation of *Anopheles gambiae* molecular forms. Malar J, 7:163.
[1] Huynh LY, Sandve SR, Hannan LM, Van Ert M, Gimnig JE. 2007. Fitness costs of pyrethroid insecticide resistance in *Anopheles gambiae*. Annual Meeting of the Society for the Study of Evolution, Christchurch, New Zealand.

done in the field in 2019 by VectorLink and National Malaria Control Program (NMCP) entomologists using the key of Gillies and Coetzee (1987). Subsequent PCR for species identification was done by LBMA on samples of *An. gambiae* s.l. collected by monthly human landing catches (HLC) and pyrethrum spray catches (PSC) in Djenné, Mopti, Bandiagara and Tominian. A sample of equal numbers of susceptible and resistant mosquitoes used for insecticide susceptibility testing were analyzed by PCR for molecular species identification and to determine the frequency of the L1014F mutation (formerly *kdr*-w) and L1014S mutation (formerly *kdr-e*) associated with pyrethroid resistance in the three IRS sites (Djenné, Mopti and Bandiagara) as well as an additional 10 sites (Koulikoro, Kadiolo, Bougouni, Bamako, Yanfolila, Baguineda, Bla, Niono, Kita and Kati). Testing for the presence of the ace-1R mutation was also planned for the 3 IRS sites (Djenné, Bandiagara and Mopti) due to the use of Actellic 300CS (organophosphate) since 2017 (in rotation with clothianidin since 2019). The number of *An. gambiae* s.l. analyzed by each method in each sentinel site is shown in Table 2.

Table 2: Number of *An. gambiae* s.l. samples analyzed by LBMA from monthly surveillance in three IRS Sites, one control unsprayed site and sentinel sites for insecticide resistance monitoring.

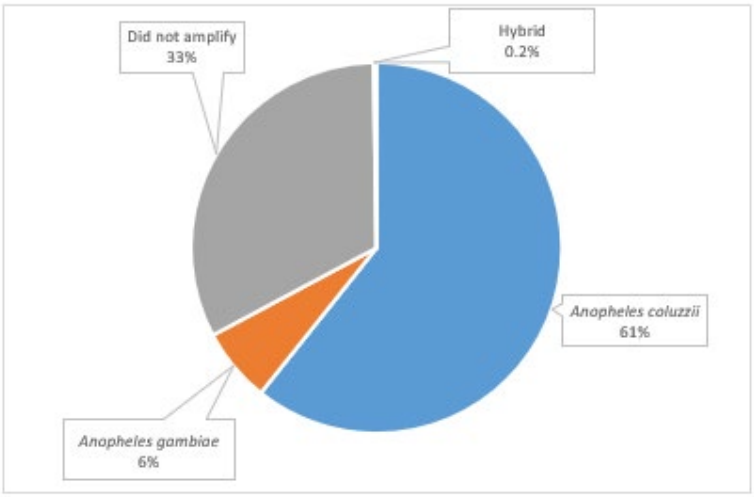
Region	Site (District)	WHO pyrethroid susceptibility test species ID	WHO pyrethroid susceptibility kdr frequency (L1014F/L)	WHO pirimiphos-methyl/ susceptibility Ace-1 frequency	HLC species ID monthly monitoring	Total tested
		# tested / # planned				
Kayes	Kita	75/75	50/50	0/0	0/0	125
	Kayes	75/75	50/50	0/0	0/0	125
Koulikoro	Koulikoro	75/75	50/50	0/0	0/0	125
	Kati	75/75	50/50	0/0	0/0	125
Segou	Niono	75/75	50/50	0/0	0/0	125
	Bla	75/75	50/50	0/0	0/0	125
	Tominian	0/0	0/0	0/0	342/182	342
Sikasso	Bougouni	75/75	50/50	0/0	0/0	125
	Yanfolila	75/75	50/50	0/0	0/0	125
	Kadiolo	75/75	50/50	0/0	0/0	125
Mopti	Mopti	75/75	50/50	0/50	137/182	262
	Bandiagara	75/75	50/50	0/50	131/182	256
	Djenné	75/75	50/50	0/50	192/182	317
Bamako	Commune iv	75/75	50/50	0/0	0/0	125
Total		**975/975**	**650/650**	**0/150**	**802/728**	**2,427**

3. RESULTS

3.1 MOLECULAR SPECIES IDENTIFICATION OF THE *AN. GAMBIAE* SPECIES COMPLEX

Out of 802 *An. gambiae* s.l. from the longitudinal entomological monitoring (3 IRS sites and one control site) analyzed, 61% were identified as *An. coluzzii.* (n = 487), 6% as *An. gambiae* (n = 52), 0.2% were hybrids (*An. coluzzii*/*An. gambiae*, n = 2) and 33% did not amplify (n =261) (Figure 2). Example PCR gel images for Djenné, Tominian and Mopti are shown in Figure A1 (Annex).

Figure 2: Molecular species composition of *An. gambiae* s.l. from 3 IRS sites (Djenné, Mopti & Bandiagara) and one control site (Tominian).

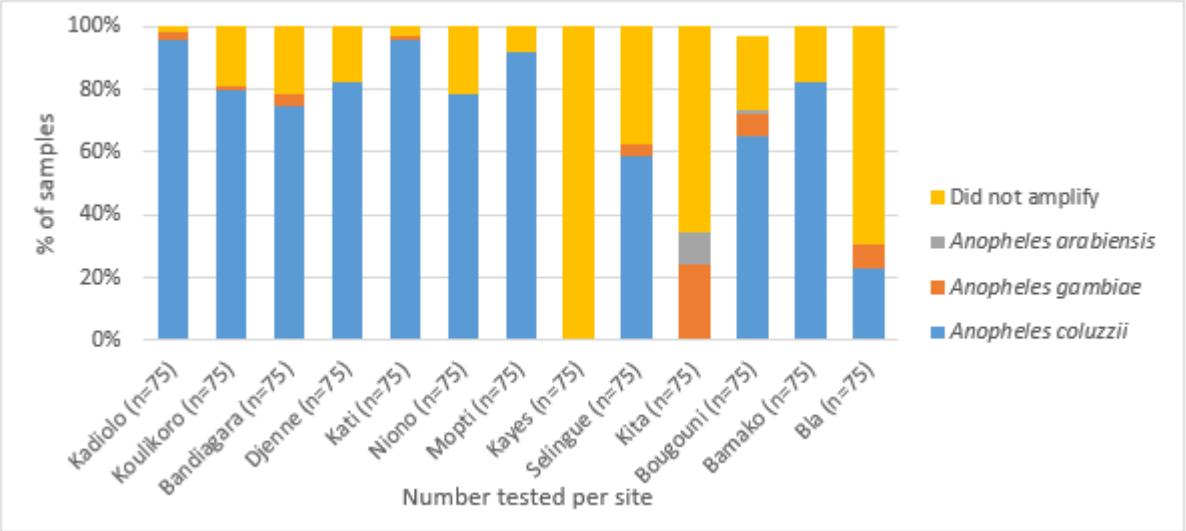


The proportion of each species per site is shown in Table 3 (collected by HLC) and Figure 3 (WHO susceptibility tests). Samples from monthly HLC and PSC collections in the three IRS sites and one unsprayed control site showed no seasonal variation in species composition. *An. arabiensis* was not detected in any of the four sites monitored. Hybrid (*An. gambiae*/*An. coluzzii*) was only recorded in Djenné at a very low frequency (1%) (Table 3).

Table 3: Molecular species composition of *An. gambiae* s.l. collected by HLC in 2019

Site	*An. coluzzii*	*An. gambiae* s.s.	Hybrid *An. gambiae*/*An. coluzzii*	*An. arabiensis*	Did not amplify	Total
Mopti	72 (53%)	6 (4%)	0	0	59 (43%)	137
Djenné	131 (68%)	9 (5%)	2 (1%)	0	50 (26%)	192
Bandiagara	69 (53%)	32 (24%)	0	0	30 (23%)	131
Tominian	215 (63%)	5 (1%)	0	0	122 (36%)	342
Total	**487 (61%)**	**52 (6%)**	**2 (<1%)**	**0 (0%)**	**261 (33%)**	**802**

Figure 3: Molecular species composition of *An. gambiae* s.l. collected as larvae for WHO susceptibility tests, 2019.



Anopheles coluzzii was the predominant species used in WHO susceptibility tests (collected as larvae) in 10 of 13 sites, while non-amplification rates were too high to determine species composition for Kayes, Kita, and Bla (65-100%). *Anopheles arabiensis* was only present Kita (11%) and Bougouni (1%). The number of samples that did not amplify varied from 1% in Kadiolo to 100% in Kayes. VectorLink targets an amplification rate of >95%, but it was <95% in 11 of the 13 susceptibility test sites and all 4 monthly monitoring sites. Example PCR gel images for samples from Kayes, Bla, Kita, Kadiolo, Sélingué and Kati are shown in Figure A2 (Annex). Further information and proposed solutions for the high non-amplification rate are included in the discussion section.

3.2 FREQUENCY OF THE *VGSC*-1014F, 1014F AND ACE-1 ALLELES

From a total of 650 *An. gambiae* s.l. specimens analyzed 77 (12%) did not amplify, 179 (28%) were homozygous (RR) for *Vgsc*-1014F, while 195 (30%) were heterozygous (RS) and 198 (30%) were wild type (SS) (Table 4). The *Vgsc*-1014S allele was absent from all samples analyzed in all the 13 sites monitored. Ace[1] analysis was not yet performed by LBMA due to a delay of reagents delivery, failure of PCR machine and COVID 19 limitation access of laboratory.

Table 4: Frequency of the *Vgsc* mutation 1014F (formerly kdr-west) in *An. gambiae* s.s. and *An. coluzzii*.

Site	Number tested	*RR*	*RS*	SS	Did not amplify	Frequency 1014F (for those that amplified)
Kadiolo	50	6	34	2	8	0.8
Koulikoro	50	38	8	4	0	0.9
Bandiagara	50	45	1	0	3	0.9
Djenné	50	9	21	18	2	0.6
Kati	50	7	18	23	2	0.5
Niono	50	0	0	50	0	0.0
Mopti	50	11	27	3	9	0.8

Site	Number tested	*RR*	*RS*	SS	Did not amplify	Frequency 1014F (for those that amplified)
Kayes	50	1	19	25	5	0.4
Selingue	50	1	12	35	2	0.3
Kita	50	20	14	7	9	0.7
Bougouni	50	13	5	19	13	0.4
Bamako	50	4	30	9	7	0.7
Bla	50	24	6	3	17	0.6
Overall	**650**	**179**	**195**	**198**	**77**	**0.6**

Note: RR=homozygous resistant, RS=heterozygote resistant, SS=homozygous sensitive.

4. DISCUSSION

Molecular analyses revealed that *An. coluzzii* was the predominant species followed by *An. gambiae* across all IRS sites (Djénné, Mopti and Bandiagara) and in the control site of Tominian. Analysis of samples from susceptibility tests also showed a predominance of *An. coluzzii*, followed by *An. gambiae,* with the exception of Kita where *An. coluzzii* was absent among identified species. In the Kayes site, none of the 75 samples amplified, while in 2019 most samples from Kayes were *An. arabiensis*. The *Vgsc*-1014F mutation was detected with a high allele frequency in 8 of the 13 sentinel sites monitored. However, in Niono, Baguenida, Selingue, Bougouni and Kayes the *Vgsc*-1014F frequency was lower than 50%. This mutation is likely to contribute to pyrethroid resistance together with other metabolic mechanisms that were implicated in synergist assays with piperonyl butoxide (PBO).

The high percentage of samples that did not amplify for species identification is very concerning. PCR gel images for all tests were shared by LBMA and the positive control samples for *An. gambiae*, *An. arabiensis* and *An. coluzzii* did amplify. We noted that non-amplification rates were lower for *Vgsc*-1014F analysis than for species identification, indicating that quantity of extracted DNA is unlikely to be the main issue (Figure A3, Annex). For example, zero samples amplified from Kayes for species identification but 95% amplified from the same site for *Vgsc*-1014F analysis. Another potential reason for low species amplification could be incorrect morphological identification of *An. gambiae* s.l. We consider this unlikely to be a major factor, however, to minimize the risk of this VectorLink Mali and LBMA have added a second level of morphological identification confirmation focused on the specific subsamples to be sent monthly to LBMA. The third round of morphological identification will be done by the LBMA technician before conducted extraction. VectorLink will work with CDC and LBMA to continue troubleshooting the reasons for these quality issues and develop appropriate quality control solutions accordingly.

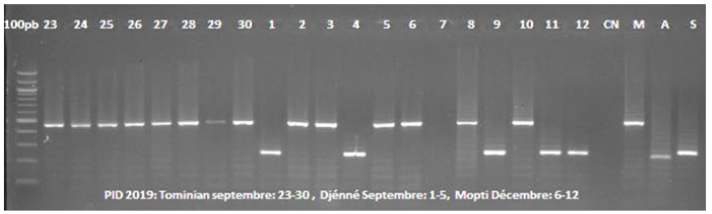
5. Annex

Figure A1. Example of gel images from *An. gambiae* species complex PCR conducted for samples from HLC according to the protocol of Santolamazza et al (2008).

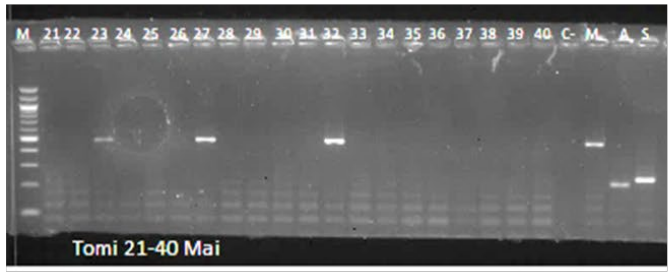
Key
Positive controls
M = *An. coluzzii*
A = *An. arabiensis*
S = *An. gambiae* s.s.
Negative control
C- = Blank

Djenné August species ID (from HLC)

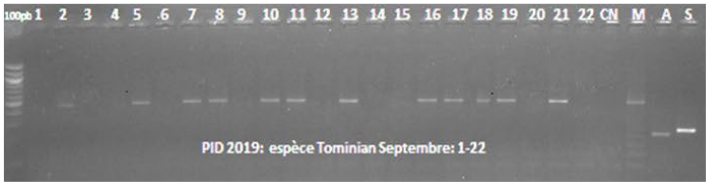
Tominian, Djenne, Mopti December species ID (from HLC)



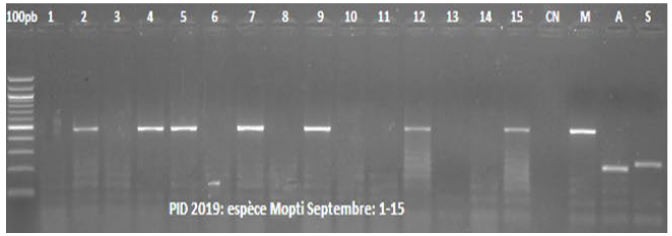
Tominian May species ID (from HLC)



Tominian September species ID (from HLC)



Mopti September species ID (from HLC)



Mopti October species ID (from HLC)

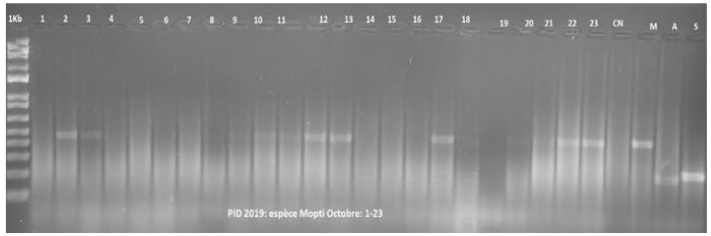
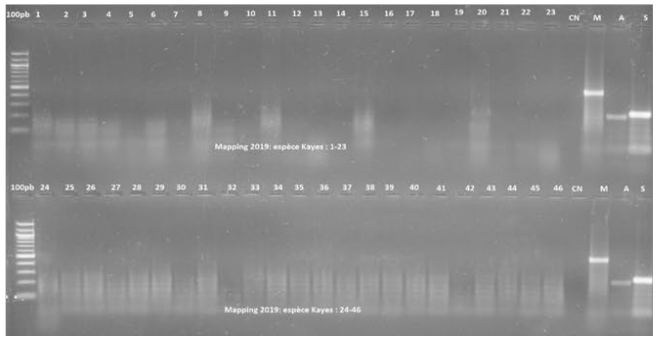


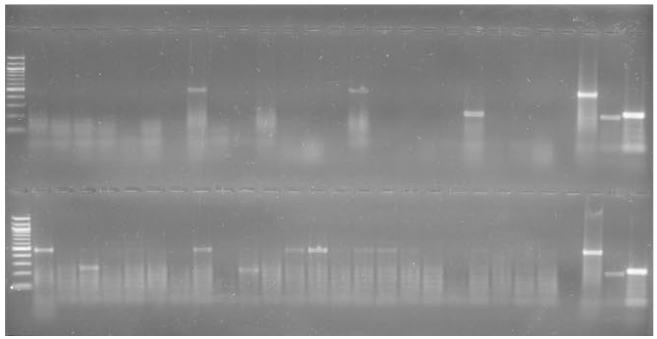
Figure A2. Example of gel images from *An. gambiae* species complex PCR conducted for samples from susceptibility tests according to the protocol of Santolamazza et al (2008).

Key
Positive controls
M = *An. coluzzii*
A = *An. arabiensis*
S = *An. gambiae* s.s.
Negative control
C- = Blank

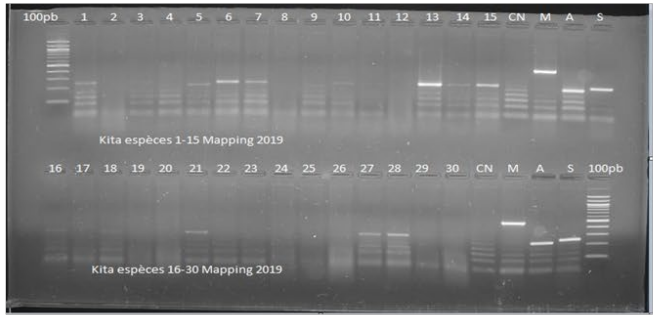
Kayes species ID (from susceptibility tests)



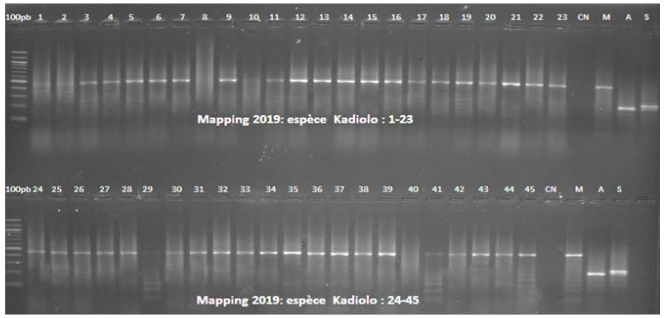
Bla species ID (from susceptibility tests)



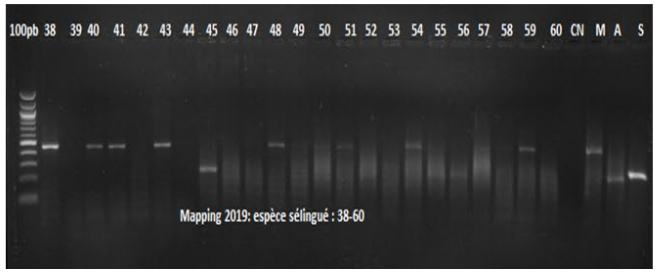
Kita species ID (from susceptibility tests)



Kadiolo species ID (from susceptibility tests)



Selingue species ID (from susceptibility tests)



Kati species ID (from susceptibility tests)

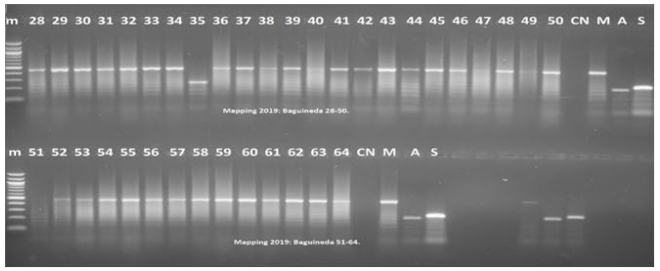
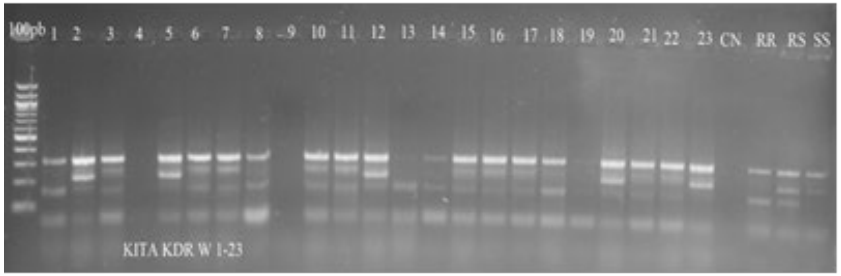
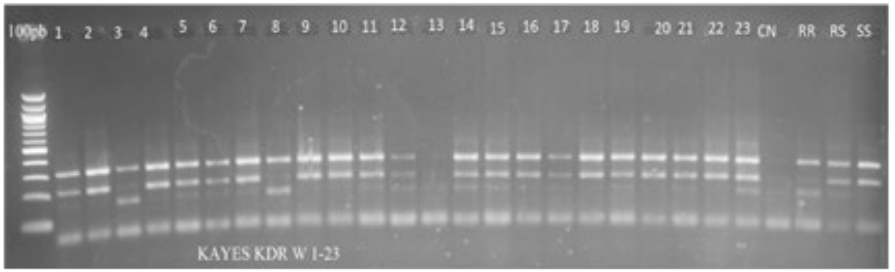
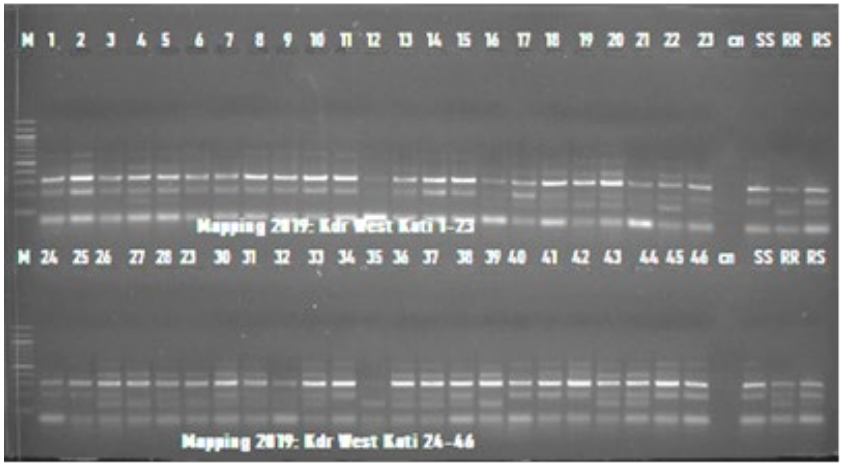


Figure A3. Example of gel images from *An. gambiae* s.l Kdr-w 1014F tests conducted for samples from susceptibility monitoring according to the protocol of Huynh et al. (2007).





KATI



NIONO