REPUBLIC OF GUINEA-BISSAU

Ministry of Public Health

Bandim Health Project

National Survey on Malaria Indicators

(MIS2023)

Final report

Funded by: Global Malaria Fund through UNDP Guinea-Bissau

The Global Fund To Fight AIDS, Tuberculosis and Malaria

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Index

ACRONYMS AND ABBREVIATIONS

AF	Households
CAP	Knowledge, Attitudes and Practices
CMILDA	MII Mass Distribution Campaign
HRP2	Histidine-rich protein II
IC	95% Confidence Interval
INASA	National Institute of Public Health
MEPIR	Ministry of Economy, Planning and Regional Integration
MICS	Multiple Indicator Survey
THOUSAND	Insecticide-Impregnated Mosquito Net
MILDA	Long-Acting Insecticide-Impregnated Mosquito Net
MINSAP	Ministry of Public Health
MIS	Survey on Malaria Indicators
OR	Odds Ratio
Pf	Plasmodium falciparum
PNLP	National Malaria Control Programme
PSB	Bandim Health Project
qPCR	Quantitative Polymerase chain reaction
FPS	Seasonal chemoprophylaxis
SAT	Bissau Autonomous Sector
SIS	Health Information System
SP	Sulfadoxine-pyrimethamine
TDR	Malaria Rapid Diagnostic Test
ICC	Intermittent Preventive Treatment in Pregnant Women

EXECUTIVE SUMMARY

The National Malaria Control Programme of the Ministry of Health of Guinea-Bissau with the support of partners, namely the Global Fund to Fight Malaria, Tuberculosis and AIDS, has intensified malaria control actions by reinforcing strategies such as early diagnosis and correct treatment, prevention through the use of insecticide-impregnated bed nets (IMI) in routine and through triennial mass distribution campaigns, preventive treatment of pregnant women and seasonal chemoprophylaxis in children <5 years old.

The Malaria Indicators Survey was conducted from October to November 2023 in 250 randomly selected clusters and 4994 households where specific questionnaires were administered, observations and health card checks performed, and the prevalence of malaria infection was determined using the CareStartTM Malaria Pf rapid test (HRP2) Ag RDT and quantitative polymerase chain reaction (qPCR).

A total of 41,304 individuals were included, of which 61% were from rural areas. The availability of insecticide-impregnated bed nets was as follows: 97% (CI:96-98) of households had at least one MII, 77% (CI:74-79) had at least one MII for every two people, and the ratio of residents to MII was 1.7. The proportion of the population with access to an MII in PA assuming that each MII of PA is used by every two people was 71% (CI: 68-73).

Regarding the use of IIM, 87% (CI: 86-89) of the general population slept under an IIM the night before, 88% (CI: 86-89) of children under five years of age and 91% (CI: 87-93) of pregnant women. In households that have at least one MII for every two persons, the use by the general population was 91% (CI:90-92). All regions had utilization levels of at least 80%.

The proportion of pregnant women who took at least three doses of intermittent preventive treatment was 54% (CI:51-57).

The prevalence of malaria infection in the community evaluated in 4300 children aged 6-59 months was 1.6% (CI: 1.1-2.2) and in the 6117 individuals ≥ 5 years it

was 5.0% (CI: 4.1-6.1). Prevalence decreased significantly from 2020 to 2023 in all age groups.

The risk factors independently associated with higher prevalence were the region of Gabú (OR=8.16; 3.81-17.5; $p<0.00$) and Bafatá (OR=3.30; 1.42-7.67; $p<0.01$), age groups over five years ($p<0.00$), reported fever and use of antimalarial drugs in the last two weeks (OR=2.50; 1.48-4.22; $p<0.01$). Schooling (OR=0.67; 0.48-0.94; $p=0.02$) was associated with lower prevalence.

Progress has been observed in malaria control indicators. It is important to reinforce ongoing actions, but also to closely monitor the epidemiological situation and barriers in order to outline specific interventions.

SUMMARY OF THE MAIN INDICATORS

Indicator	Value (year)	MIS 2012	MIS 2014	MIS 2017	MIS 2020	MIS2023
Proportion of households that have at least one MII	47% (2010)*	99%	97%	98%	93%	97%
Ratio of MII by persons in households	-	2.3	1.9	2.0	2.4	1.7
Proportion of households that have at least 1 MII to two people (Malaria O-4.1)	-	-	78%	70%	63%	77%
Proportion of population with access to an MII in the household (who slept the previous night in a household with at least one MII for every two people) (Malaria O-2)	-	-	-	-	43%	71%
Proportion of Population That Slept Under an MII the Preceding Night (Malaria O-1a)	-	93%	81%	92%	84%	87%
Proportion of Children Under Five Who Slept Under an MII the Previous Night (Malaria O-1b)	36% (2010)*	94%	82%	92%	89%	88%
Proportion of pregnant women who slept under an IIM the night before (Malaria O-1c)	32% (2010)*	91%	84%	92%	88%	91%
Proportion of the population that slept under an MII the night before in households with at least one MII	-	-	-	-	87%	88%
Proportion of the population that slept under an MII the night before in households with at least one MII for every two people	-	-	-	-	91%	82%
Proportion of the population that slept the night before in a household with at least one MII for every two people	-	-	-	-	43%	71%
Proportion of TPI-3 or more in pregnant women (SPI-1)	-	-	7.5%	9.5%	45%	54%
Prevalence of malaria infection: proportion of the population with infection (Malaria I-5.1)	-	-	-	-	6.4%	3.1%
Prevalence of malaria infection in children 6-59 months of age (Malaria I-5.1)	-	9.9%	1.3%	0.7%	3.6%	1.6%
Prevalence of malaria infection in individuals aged 5 years and older (Malaria I-5.1)	-	7.9%	0.7%	1.5%	7.8%	5.0%

* MICS-4

1. INTRODUCTION

1.1. Guinea-Bissau

Guinea-Bissau is located in West Africa between 11 ∘ 52′N and 15 ∘ 36′W, has just over two million inhabitants according to projections from the national population census carried out in 2009 (INE 2009). The country is subdivided into eight administrative regions and an autonomous sector, subdivided into sectors. The health organization distinguishes eleven health regions, namely: Bafatá, Bijagós, Biombo, Bolama, Cacheu, Gabú, Farim, Oio, Quinara, SAB and Tombali. There are more than twenty ethnic groups, the most numerous being the Fula, Balanta, Mandinga, Pepel and Manjaca ethnic groups (Figure 1).

Figure 1. Map of Guinea-Bissau and regions

The climate is tropical, hot and humid, with two seasons: the dry season and the rainy season, with the rainy season being from mid-May to mid-November, with greater rainfall in July and August.

Guinea-Bissau ranks 179th out of 193 countries in terms of the Human Development Index and the population living below the monetary poverty line is 21.7%, according to the UNDP's 2023 Human Development report.

1.2. Malaria in Guinea-Bissau

The World Health Organization (WHO) estimated 249 million malaria cases and 608,000 deaths in 2022 in the 2023 World Malaria Report. The WHO African Region accounted for 94% of the cases.

In Guinea-Bissau, according to the Annual Report of the National Malaria Control Program (PNLP) of the Ministry of Health of Guinea-Bissau, a total of 181,855 cases of malaria were registered in 2021, constituting 24% of all consultations and 466 deaths, with 26,713 (15%) cases and 95 (21%) deaths in children under five years of age.

Malaria is endemic in Guinea-Bissau with stable transmission throughout the country and with an increase in cases in the rainy season. The most common parasite is *Plasmodium falciparum* , which makes up about 98% of infections. The most common mosquito species is *Anopheles gambiae sensu strictu, An. melas* in coastal and river zones, *An*. coluzzi, *An*. Arabienses, with moderate to high resistance to permethrin and deltamethrin detected (Nwakanma 2013; Gordicho 2014; INASA 2017; Silva, 2020).

With regard to prevention indicators, 93% of households had at least one IIM in 2020, with the use of around 84%, and 45% of pregnant women who attended at least one antenatal visit received ICT3. Seasonal malaria chemoprophylaxis in children aged 3-59 months in the regions of Bafatá and Gabú has reached administrative coverage of more than 85%.

The strategies of the National Malaria Programme are aligned with the Global Technical Malaria Strategy 2016-2030 (GTS) adopted by the 68th World Health Assembly in May 2015. With the support of partners, namely the Global Fund to Fight Malaria, Tuberculosis and AIDS (FM), the PNLP has intensified malaria control actions by reinforcing strategies such as early diagnosis and correct treatment, prevention through the use of insecticide-impregnated bed nets that do not require re-impregnation (MII), preventive treatment of pregnant women and seasonal chemoprophylaxis in children under five years of age.

Since November 2011, mass distribution campaigns of MII (CMILDA) have been carried out to the entire population every three years, in addition to the routine distribution in prenatal consultations and in the Extended Vaccination Program for children. In June

2023, the last CMILDA was held, with MII with PBO being distributed in the regions of Bafatá, Gabú, Tombali and Bolama. Intermittent preventive treatment (IPT) with sulfadoxine-pyrimethamine in pregnant women has been adopted since 2004 with the offer of at least three doses. Seasonal malaria chemoprophylaxis (QPS) for children aged 3-59 months in the months of August to November was adopted in 2016 in the regions of Bafatá and Gabú, and was extended in 2020 to the Bolama and Tombali regions.

2. GOALS

The general objective of this MIS survey was to estimate the main malaria indicators at the household level in order to provide subsidies for the evaluation of the results of malaria control and the definition of evidence-based control strategies and actions.

The specific objectives were:

a) Assess the availability of MII in households at national level, by health region, sector and urban or rural means of residence;

b) Estimate the use of insecticide-impregnated bed nets by the general population, children under five years of age and pregnant women at national level, by health region, sector and urban or rural means of residence;

c) To estimate the prevalence of malaria parasitemia in the community in children aged 6 to 59 months and in those over 5 years of age;

d) To estimate the prevalence of the occurrence of fever in the community in children under five years of age, the search for treatment, the performance of laboratory tests and antimalarial treatment;

e) To estimate the proportion of pregnant women who received at least three times the intermittent presumptive treatment with PS;

f) Assess knowledge, attitudes and practices (CAP) regarding malaria.

3. METHODS AND ORGANIZATION

The standard methods and instruments recommended in the April 2018 Household Survey Indicators for Malaria Control for conducting these malaria indicator (MIS) surveys, also with reference to the strategic plan of the PNLP of Guinea-Bissau, the monitoring and performance framework of the Global Fund, recommendations of the WHO, Roll Back Malaria (RBM) and the Monitoring and Evaluation Reference Group of the RBM.

3.1. Study design, site, and sample

This is a cross-sectional survey by cluster in households in all 11 health regions of Guinea-Bissau: Bafatá, Bijagós, Biombo, Bolama, Cacheu, Gabú, Farim, Oio, Quinara, Autonomous Sector of Bissau (SAB) and Tombali (Figure 1).

The sample was taken in two steps, selecting the clusters, each of which was a domain. The sample was selected in a stratified manner by each sector in the region in a proportional manner. The target was the entire population in the selected households and specific population groups depending on the indicator (Table 1).

Table 1. Population included in the study according to indicators

Indicator Group	**Target population/respondent**	**Method/procedure**
Socioeconomic characteristic	Adult Household Representative	Interview via standard questionnaire
Knowledge, attitudes and practices	Adult Household Representative	Interview via standard questionnaire
Availability of MII	Household/All residents	Interview and observation through standard questionnaire
Use of MII	Residents who spent the previous night at the site	Interview and observation through standard questionnaire
Prevalence of malaria parasitemia	All gifts from 6-59 months of age; Random sample of gifts ≥ 5 years old	TDR and filter paper for qPCR
Occurrence of fever, seeking health services and medication	All gifts from 0-59 months of age; Participants ≥ 5 years of age in the prevalence study	Interview via standard questionnaire

Seasonal chemoprophylaxis coverage	Children residing from 3-59 months of age in the regions of Bafata, Gabu, Tombali and Bolama	Interview through standard questionnaire and QPS card inspection
Intermittent preventive treatment in pregnant women (TPI)	Women who have given birth in the last 24 months	Interview through standard questionnaire and prenatal consultation card inspection

The sample size was calculated in Stata based on the prevalence of parasitemia observed in the 2017 survey, as it is the indicator with the lowest numerical value. Thus, assuming a prevalence of 1.3% in children aged 6-59 months with alternative prevalences (precision) of 1.88%, power of 80%, 95% confidence interval, and multiplying by the *deff* coefficient of 1.5 for correction by the design effect by cluster, we obtained about 4900 from 6-59 months and approximately the same for children over 5 years of age. 250 clusters were selected in proportion to the population of the region and sector, but with some adjustments in order to allow better representation of small regions; In each cluster, 20 households were included.

3.2. Selection and procedures

The clusters were randomly selected in each sector from the list of localities of the last general population census of INE in 2009. The procedures in the field consisted of an initial census of all households in rural areas, assigning current numbers, collecting GPS and the name of the head of household or other reference that would facilitate the return to FA if selected. Using a table of random numbers, they selected 20 AFs and 10 substitute AFs in case of refusal or absence of eligible responders using them. In urban areas, the 20 AFs and 10 additional supplements were randomly selected and the coordinates included in the maps of the respective conglomerates available on the android devices used for data collection, orienting themselves through GPS to reach the point.

In the households that consented to participate, all rooms were visited and the residents of each bed were registered, as well as the verification of MII through the brand. Each participant included in one of the studies was assigned a barcode generated in advance by a program and pre-printed on a label that was scanned on the tablet or smartphone using the *Barcode Scanner app*. The questionnaires in Portuguese were applied in Creole or, in certain cases, in ethnic languages.

Laboratory procedures included performing the ACCESS BIO CareStartTM Malaria Pf (HRP2) Ag RDT rapid malaria test (RDT) for antigen detection of *Plasmodium falciparum*. Drops of blood on filter paper (DBS) were also taken to perform quantitative polymerase chain reaction (qPCR) for ultra-sensitive detection of *Plasmodium falciparum* (Hofmann et al., 2015) (see detailed description in Annex 1).

3.3. Data management and analysis

The data were collected on android devices using Open Data Kit (ODK), GeoODK version with the added value of capturing and visualizing coordinates on the map. On a daily basis, the collected data was sent to a copy of ODK (ODK Aggregate) installed on a *cloud* platform using encrypted connections (SSL) and access to ODK Aggregate was restricted to the use of user codes and passwords. Quality control was done at all stages through logical verification and coherence, triangulation of responses and completion of information, being facilitated by the use of filters and logical controls in ODK. Data analysis was performed in Stata 18.0.

The proportions and confidence intervals of the indicators were calculated at the national level and by health region, weighted by the regional population and taking into account the cluster design. The association between key indicators (use of IIM, malaria prevalence) and region, sex, age group, and urban or rural area of residence was explored using the X2 test and logistic regression models to adjust for various factors.

All mosquito nets impregnated in the factory were considered impregnated mosquito nets, regardless of how many years of use, so even those distributed in the 2020 campaigns, or previous or by the PAV CPN years ago were considered MII.

4. ETHICAL CONSIDERATIONS

The survey protocol was approved by the National Health Ethics Committee (Ref.:032/CNES/INASA/2023). Prior to inclusion, participants were asked for oral consent after explanation of the reasons and objectives of the survey, the procedures, i.e., the interview and testing. For the children, consent was requested from the guardian.

The interviews do not contain any personal information that could cause any embarrassment. The laboratory procedures were minimally invasive, although there could be some discomfort with the finger prick, they are routine procedures in health centers and known to relatives. All biosafety standards were followed, namely the use of disposable individual lancets, the waste was collected by the team and treated properly in the nearest health centers.

All people with fever and positive RDT tested received paracetamol and were treated with COARTEM according to the PNLP policy or advised to seek a nearest health facility.

To estimate the level of economic power of households and create the socioeconomic index, the statistical method "Principal Component Analysis" (PCA) was used, using the methods developed in previous literature. Data on the main characteristics of household housing and household assets were used.

5. FINDINGS

5.1. Sample Description

Data collection took place from October 5 to November 7, 2023. 250 clusters were included, of which 61% were in rural areas. Of the 5,006 households, 12 refused to participate (5 from SAB, 3 from Oio, 2 from Cacheu, 1 from Biombo and 1 from Bafatá.

Of the 4,994 households included, 41,304 persons were registered, of whom 24,634 (60%) were present, 16,381 (40%) were absent or travelling, and 289 (0.7%) were guests; 54% were female (Table 1).

Table 1. Inclusion in the community by region.

Region	No. of conglomerate	Number of AF	No. of individuals	Absent / Travel (%)	Rural (%)	Female gender (%)	No. MIF	Pregnant (%)
Bafatá	30	600	5695	1 964 (34)	4 242 (74)	3 101 (54)	1524	121 (7,9)
Bijagós	12	240	1371	334 (24)	972 (71)	739 (54)	382	20 (5,2)
Screen	20	400	2762	1 133 (41)	1 981 (72)	1 542 (56)	780	41 (5,3)
Bolama	10	199	1446	679 (47)	1 118 (77)	777 (54)	433	11 (2,5)
Cacheu	30	600	5155	2 029 (39)	3 753 (73)	2 705 (52)	1385	76 (5,5)
Farim	12	240	2771	1 285 (46)	2 216 (80)	1 590 (57)	730	54 (7,4)
Gabú	30	599	5597	1 985 (35)	4 287 (77)	3 091 (55)	1466	109 (7,4)
Oio	26	520	4575	2 005 (44)	3 359 (73)	2 509 (55)	1206	85 (7,1)
Quinara	20	400	3182	1 220 (38)	2 312 (73)	1 676 (53)	806	59 (7,3)
SAT	40	796	5350	2 173 (41)	0 (0)	3 051 (57)	1752	75 (4,3)
Tombali	20	400	3400	1 574 (46)	2 590 (76)	1 723 (51)	906	59 (6,5)
Total	250	4 994	41 304	16 381 (40)	26 830 (65)	22 504 (54)	11 370	710 (6,2)

The median age was 18 years (interquartile range: 8 to 34 years), of which 5,925 (14%) were under five years of age, 11,663 (28%) were aged 5 to 14 years, 8,048 (19%) were between 15 and 24 years, and 15,668 (38%) were 25 years of age or older.

Of the 11,370 women aged between 12 and 50 years, 710 (6.2%) were pregnant and no information was obtained on 38 (0.3%); 2,359 (21%) reported having had a birth in the last 24 months.

The main ethnic groups included in the sample were the Fula (27%), Balanta (21%), Mandinga (18%) and Pepel (6.7%), corresponding to the main ethnic groups in the country (Figure 2).

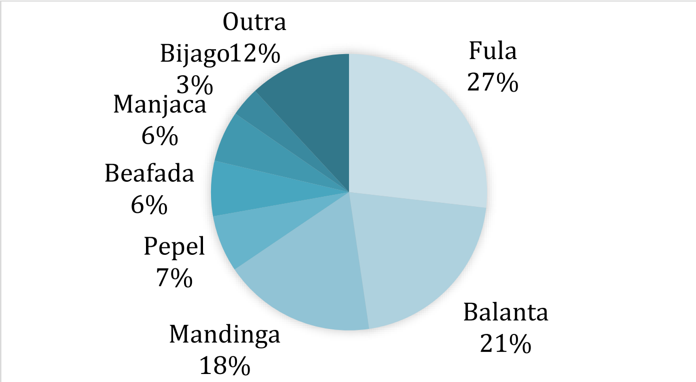

Figure 2. Ethnic distribution of the sample.

The questionnaire regarding the socioeconomic situation of the household was applied to 4945 main respondents, of which 82% were the head of the household, 60% were from a rural area, 47% were male, 39% had never attended school with the median class finishing the seventh.

Figure 3 shows the distribution of the socioeconomic status of household aggregators in terms of the socioeconomic index, a composite of variables that are relevant in describing health inequalities of PSs and can be interpreted as a relative measure of socioeconomic status in the sample. Some of the variables used are the conditions of the household's housing: water source, sanitation, floor material, roof, wall. The distribution of the index is normal, continuous, and centered on the value of zero. The index range is from -2.59 to 12.34, with a standard deviation of 1.51. The median value is -0.37.

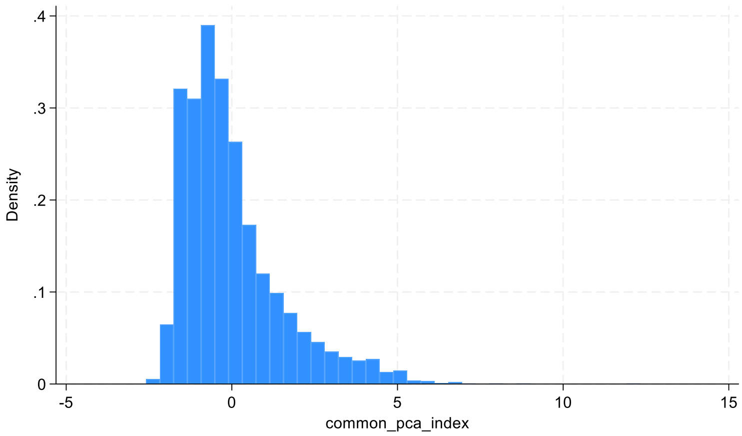

Figure 3. Density Distribution of the Socioeconomic Index.

Most families used water from a well or tap outside the house (73%) and 19% from a tap in the house itself. As for basic sanitation, 34% used a common latrine or outside the home, 21% a latrine in the house, 19% a bathroom inside the house, 17% a bathroom outside the house with a septic tank, and 8.1% did not have access to, that is, they use it outdoors. In 56% of the houses the floor was made of cement, in 33% of rammed earth and in 11% mosaic, 92% were covered with zinc, in 71% the walls were in adobe or rammed earth and in 66% they had no ceiling.

5.2. Possession and availability of MII in households

5.2.1. Characteristics of MIIs in households

During visits to households, surveyors had access to 95% of the rooms to verify whether or not there was a mosquito net and the mark on each bed. In the unvisited rooms, a knowledgeable respondent provided the information. In the rooms to which they had access, a mosquito net was seen hanging in 87% of the cases, in 6.8% it was seen folded and it was not seen in 5.9% of the beds; in cases in which they did not have access, in 88% of the cases they said they had mosquito nets, in 7.8% that they did not have them and in 4.4% they could not say (Table 2).

Table 2. Verification of the existence of mosquito nets in sleeping places (beds).

Region	Slept in AF	Total beds	Verified tent			Based on the answer		
			Hanging	Folded	Not seen	Has	There is none	Don't know
Bafatá	5495	2855	2419	136	170	116	4	10
Bijagos	1283	727	590	14	90	30	2	1
Screen	2565	1340	658	499	129	46	7	1
Bolama	1374	790	735	22	12	20	1	0
Cacheu	4780	2701	2242	100	230	104	10	15
Farim	2703	1345	1260	29	30	25	1	0
Gabú	5458	2788	2360	164	134	114	7	9
Oio	4458	2308	2129	74	46	58	1	0
Quinara	3087	1744	1594	48	31	70	1	0
SAT	5254	2685	2081	218	254	103	28	1
Tombali	3209	1784	1539	72	62	101	8	2
Total	39 666	21 067	17 607	1376	1188	787	70	39

The impregnated mosquito net marks seen during the observation were mainly *Royal Sentry in 39% of the PAs, OlysetPlus in 32% and Permanet* in 9.0%, and other MIIs (*DuraNet, MAGnet and others*) were seen in smaller quantities, but also a considerable amount (16%) of mosquito nets without the label, mainly because they joined two mosquito nets and made a single one in order to increase the size (Table 3).

Table 3. Impregnated mosquito net marks observed in households.

Region	Relative proportion of brands in each region (%)				
	Olyset Plus	Permanet	Royal Sentry	No label	Another brand
Bafata	70	1,5	3,7	17	7,9
Bijagos	68	3	13	14	2,0
Screen	0	23	61	13	3.0
Bolama	80	8,9	2,9	6,9	1,3
Cacheu	0,3	23	48	23	5,6
Farim	0	1,7	84	14	0,5
Gabu	78	6,3	4,4	7,4	4,0
Oio	0,3	2,3	64	28	5,5
Quinara	0,2	1,9	81	14	2,8
SAT	0,2	19	66	13	2,2
Tombali	73	4,0	4,0	13	5,4
Guinea-Bissau	32	9,0	39	16	4,0

With regard to the origin of the MII, of 18,354 mosquito nets with information, 14,132 (77%) came from the 2023 CMILDA campaign, 3,704 (20%) from CMILDA 2020 or earlier, 283 (1.5%) came from the CPN, 95 (0.5%) from the PAV, with the remaining 110 (0.6%) as a gift and 30 (0.2%) purchased.

The vast majority (97%) of the mosquito nets came from a mass distribution campaign of mosquito nets. It should be noted that 20% of the mosquito nets in use in the PS came from the 2020 CMILDA, and this proportion was very high in the regions of Cacheu (39%), Biombo (30%), SAB (27%) and Oio (27%) (Figure 4).

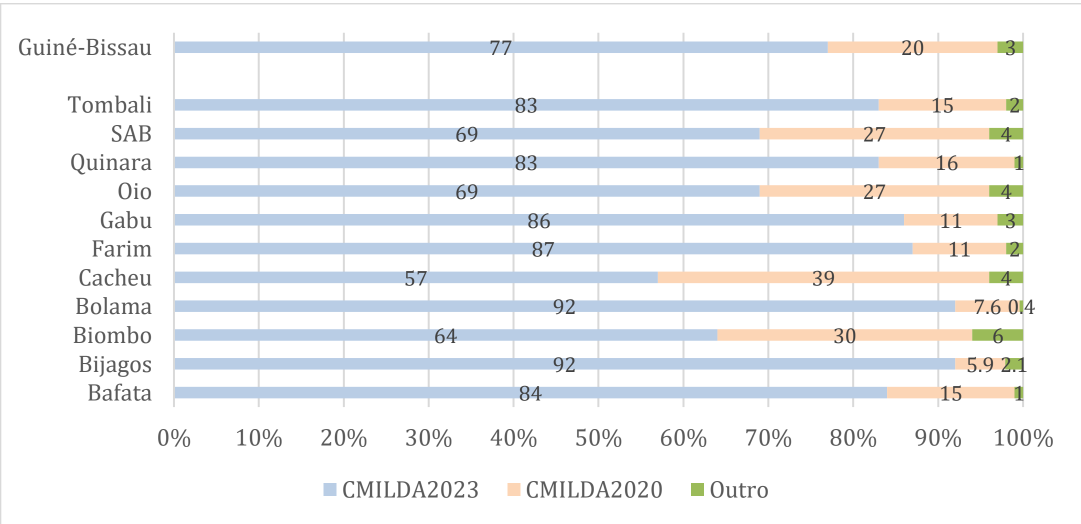

Figure 4. Origin of MIIs used in the household.

Analyzing the existence or not of MII from the last CMILDA2023 campaign or the health system unused, only 45% of households declared that they did not have any MII from the last CMILDA 2023 campaign unused, and 4.1% could not say whether they had it or not; in addition, about 34% of FAs reported having more than one new unused MII (Figure 5).

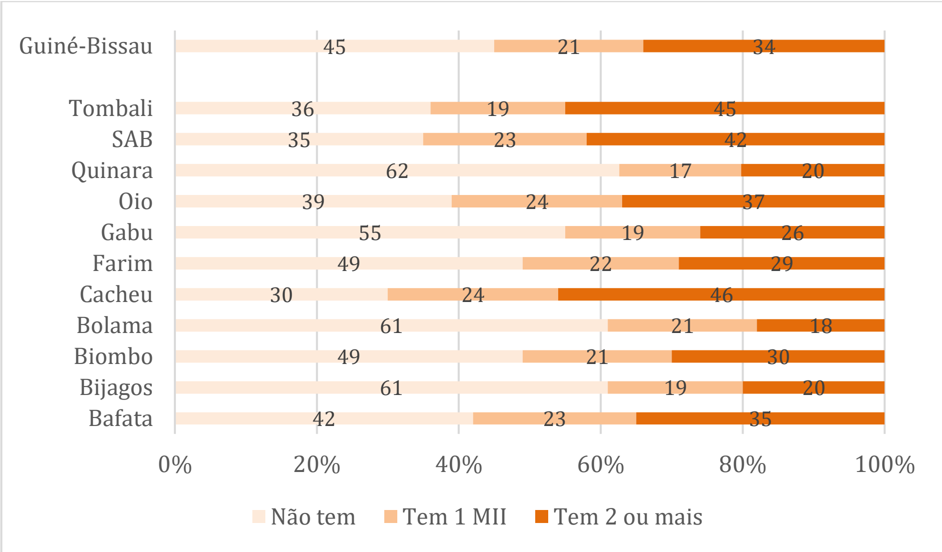

Figure 5. Existence of MII of unused CMILDA2023 in the household.

5.2.2. Availability of MILDA in households

In the analysis on the availability of MII, 4 993 households were included. The proportion of households that had at least one MII was 97% (95% Confidence Interval [CI]:96-98) and was universally high across all regions (Figure 6).

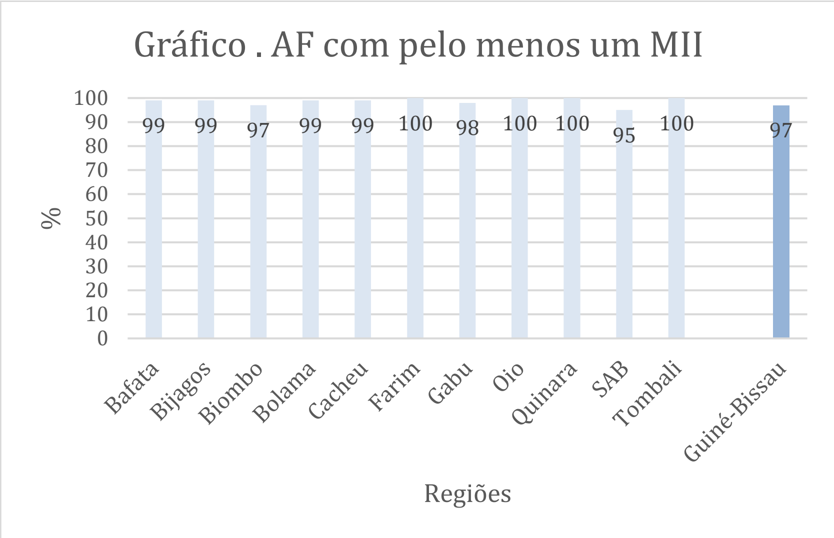

Figure 6. Proportion of AF that have at least one MII.

The proportion of households that had at least one MII for every two people who spent the previous night in the PA depicts the proportion of FAs that have a sufficient number of MIIs to cover all the individuals who spent the previous night there, assuming that an MII is used by two people, and thus describing the intra-household gap.

The proportion of PA with at least one MII for every two people who spent the previous night was 77% (CI: 74-79) and the ratio of persons in the household to MII averaged 1.7. The regions of Biombo (69%) and Gabú (69%) did not reach 70% (Figure 7).

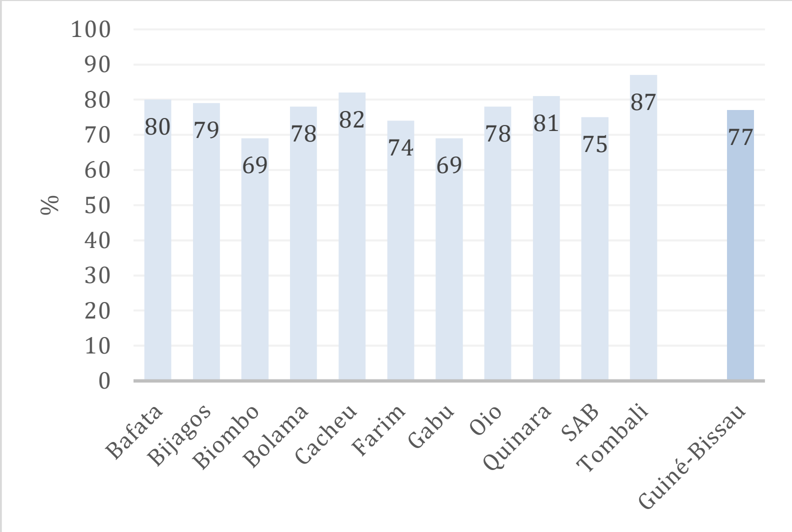

Figure 7. Proportion of AFs who have at least one MII to two people.

Access to an MII in households is measured by the proportion of population with access to MII in FA, i.e. those who spent the night and could sleep under an MII assuming that each MII of the FA is used by every two people. This proportion of the population with access to an IIM in their PA was 71% (CI: 68-73), and was lower in Biombo (58%) and Gabú (61%) (Figure 8).

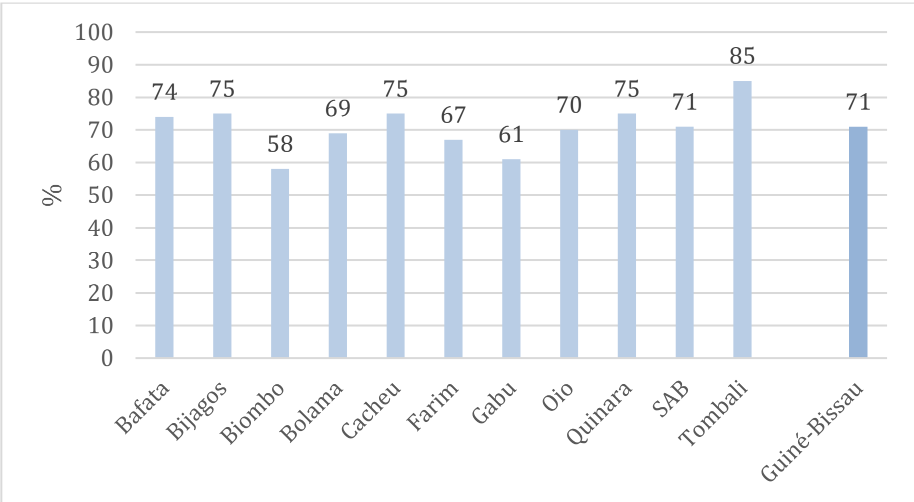

Figure 8. Proportion of the population with access to MII in their household.

5.3. Use of insecticide-impregnated mosquito net

The evaluation of the use of MII was based on 39,519 individuals who spent the previous night in the household, in whose beds it was possible to verify the existence of MII and its imprint or through responses. The proportion of IIMs in households used the previous night was 71% (CI: 70-72).

The proportion of residents, regardless of age, who slept under an IIM on the night prior to the survey was 87% (CI: 86-89), with significantly lower coverage observed in the regions of SAB (82%), Biombo (83%), Cacheu (83%) and Bolama (84%) (p<0.01) (Table 4).

Table 4. Proportion of residents who slept under MII the night before.

Region	N	n	Usage (%)	95% CI
Bafatá	5 479	5 011	91	91-93
Bijagós	1282	1091	88	82-92
Screen	2563	2150	83	79-87
Bolama	1374	1154	84	78-89
Cacheu	4748	3997	83	80-86
Farim	2703	2553	95	92-96
Gabú	5383	5008	92	86-95
Oio	4453	4112	92	89-94
Quinara	3085	2804	92	89-94
SAT	5243	4373	82	79-85
Tombali	3206	2878	88	83-92
Total	39 519	35 131	87	86-89

Note: Weighted by regional population; n=nº of those who slept under MII; N=total number of those who spent the previous night in AF.

In households that had at least one IIM, the proportion of the population that used IIM was 88% (CI:87-89), and was similar for both sexes. In FAs that had at least one MII for every two people, the use of MII by those who spent the night in PA was 91% (CI: 90-92) (Figure 9).

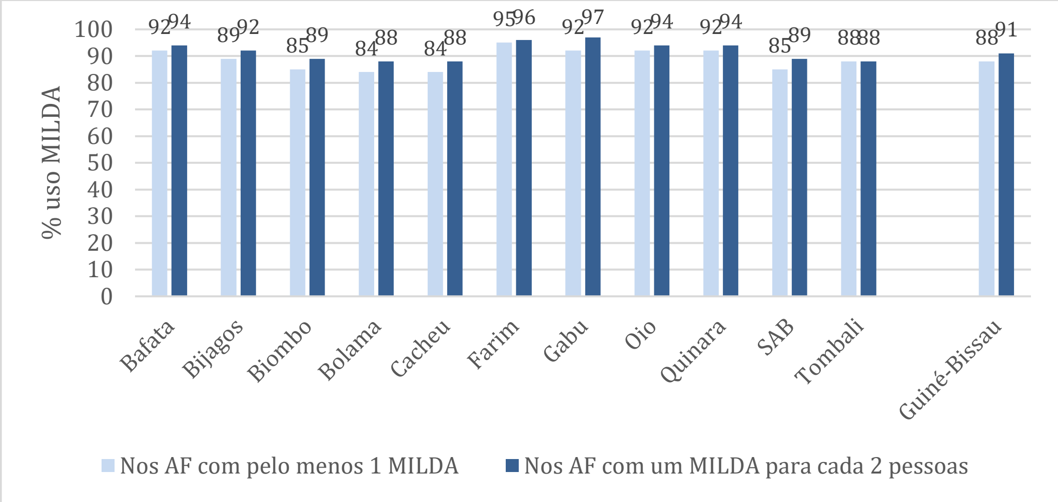

Figure 9. Use of MII in FAs that had MII at least one MII and one MII for every two people who spent the night.

As for children under 5 years of age, of the 5,757 included in the analysis, 88% (CI: 86-89) had slept under an IIM the night before. The regions with the lowest coverage were Biombo (81%), SAB (82%), Bolama (84%) and Cacheu (85%) ($p<0.02$) compared to Bafatá (Table 5).

Table 5. Proportion of children under 5 years of age who slept under MII the night before.

Region	N	n	Usage (%)	95% CI
Bafatá	891	839	94	92-96
Bijagós	159	150	97	92-99
Screen	395	326	81	73-87
Bolama	165	138	84	70-92
Cacheu	654	569	85	80-88
Farim	425	407	96	91-98
Gabú	1009	966	94	87-97
Oio	681	635	93	90-95
Quinara	422	381	92	87-95
SAT	537	445	82	78-85
Tombali	415	385	91	80-96
Total	5753	5241	88	86-89

**Weighted by the regional population; n=nº of those who slept under MII; N=total no. of 0- 59 months*

Of the 678 pregnant women included, the proportion of those who had slept under an IIM the previous night was 91% (CI: 87-93) (Table 6).

Table 6. Proportion of pregnant women who slept under MII the night before

Region	N	n	Usage (%)	95% CI
Bafatá	117	106	91	80-96
Bijagós	20	20	100	-
Screen	39	36	92	77-98
Bolama	10	8	80	56-93
Cacheu	70	63	90	72-97
Farim	50	48	96	85-99
Gabú	105	100	95	89-98
Oio	82	77	94	85-98
Quinara	57	53	93	85-97
SAT	73	62	85	74-92
Tombali	55	50	91	78-97
Total	678	623	91	87-93

5.3.1. Use of MII by different determinants

An association was observed between the use of MII and the area of residence. The proportion of those who used MII the night before in urban areas (83%; CI: 80-85) was significantly lower than in rural areas (90%; CI: 89-92) (p<0.00) (Figure 10).

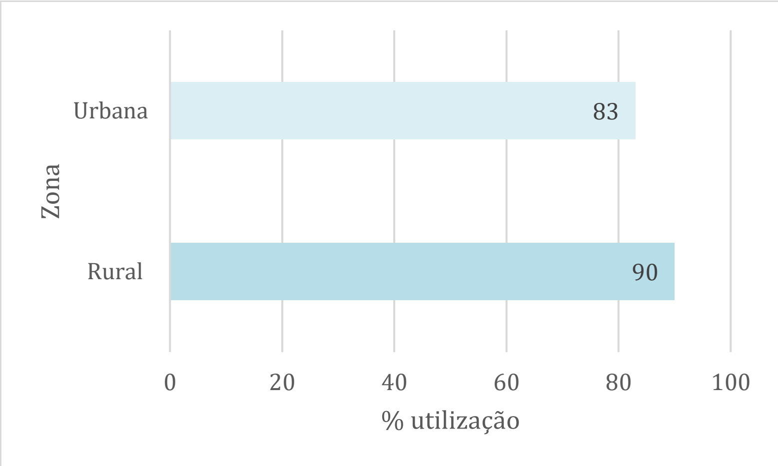

Figure 10. Proportion of individuals of all ages who slept under MII by zone.

The use of MII by individuals of all ages varied by sector, with significant disparities between sectors within certain regions. All sectors have achieved at least 80% coverage of MII utilization, with the exception of the Komo sector (78%) in the Tombali region (Figure 11).

In children under 5 years of age, disparities between sectors were also observed and the use of MII was also lower in Komo (73%).

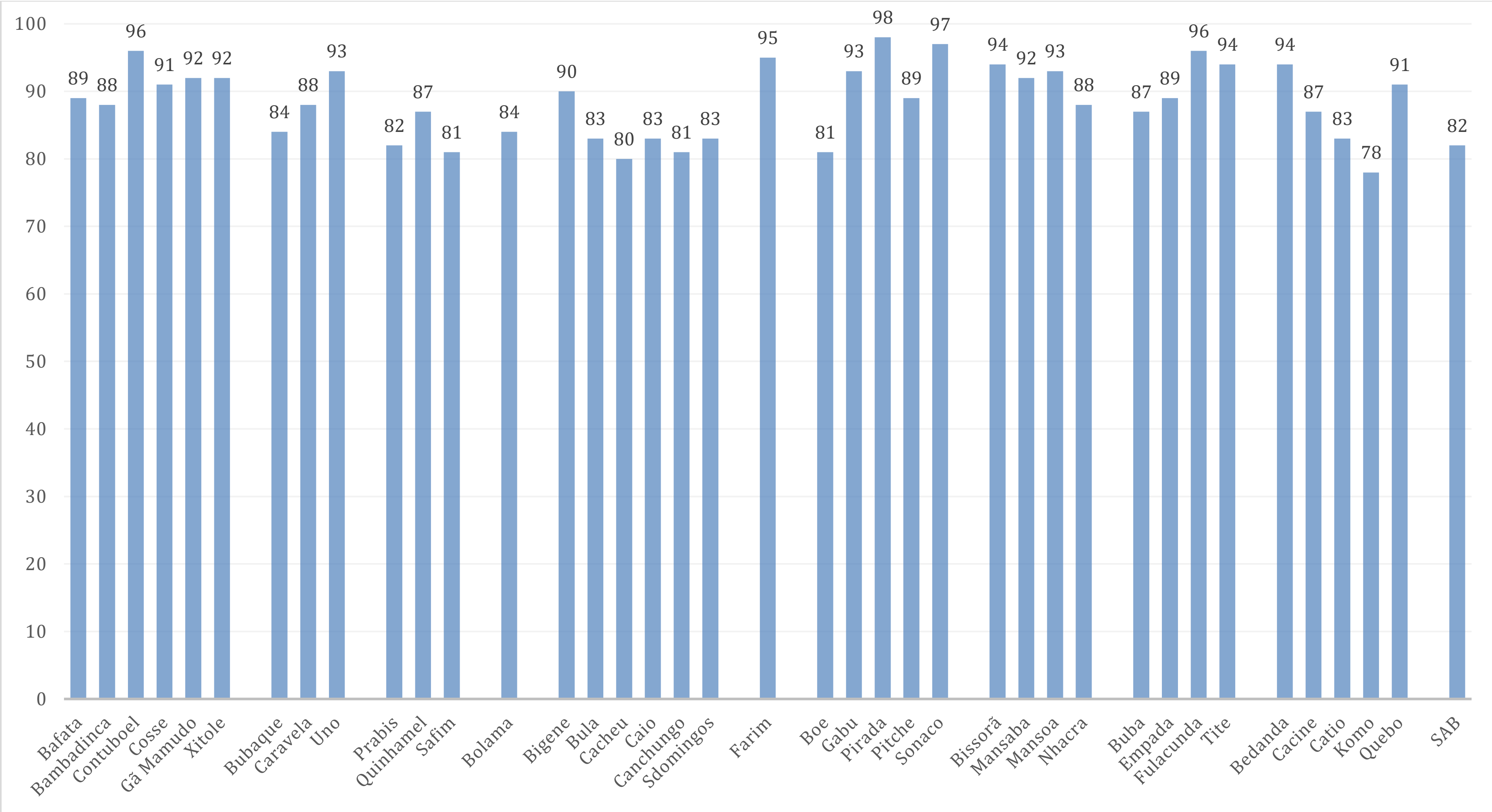

Figure 11. Proportion of individuals of all ages who slept under MII the night before by sector.

No differences were observed in the use of IIM by sex, as 86% (CI: 85-88) of females and 87% (CI: 85-88) of males used IIM (p=0.84). The age groups of those over 25 years of age and especially those over 50 years of age used the MII less (Figure 12).

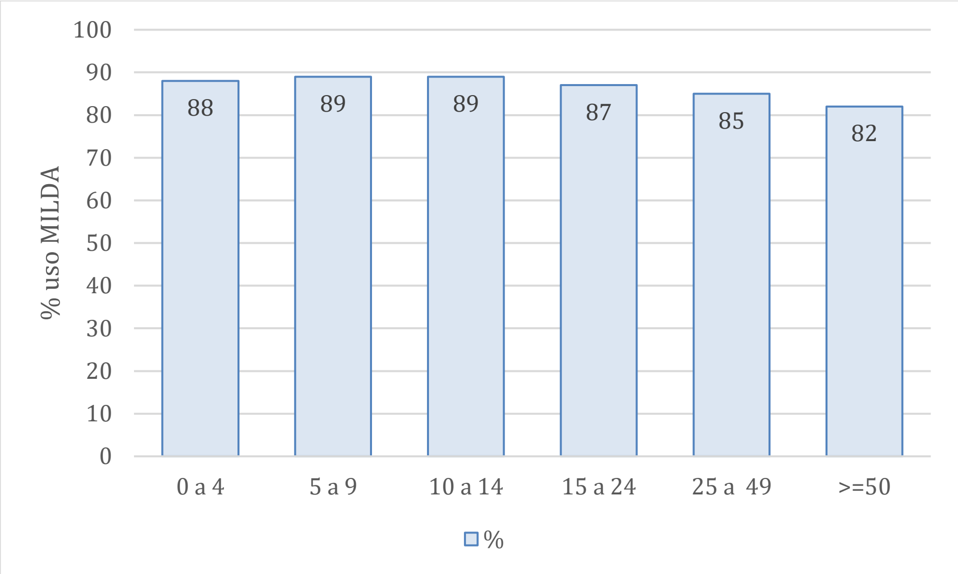

Figure 12. Proportion of MII use by age group.

The use of MII by the entire population was not different between the first levels but was significantly lower in the fifth quintile of the socioeconomic level (Table 7).

Table 7. Use of MII according to socio-economic situation

Socioeconomic index	N	n	% (95% CI)	OR (95%CI), *p-value*
1st quintile	6401	5 839	90 (87-92)	1
2nd quintile	7 185	6 579	91 (87-93)	1.13 (0.70-1.82); 0.60
3rd quintile	8 442	7 630	90 (88-91)	1.03 (0.76-1.40); 0.84
4th quintile	8 826	7 886	89 (86-91)	0.90 (0.63-1.29); 0.58
5th quintile	8 303	6 868	80 (77-82)	0.46 (0.33-0.65); <0.00

The reasons for not using IMI were mainly because they used other methods (30%) (e.g. pesticide, fan, air conditioning, repellent), did not have an IMI (25%), considered there were no mosquitoes (15%), suffocated or felt very hot (15%) (Figure 13).

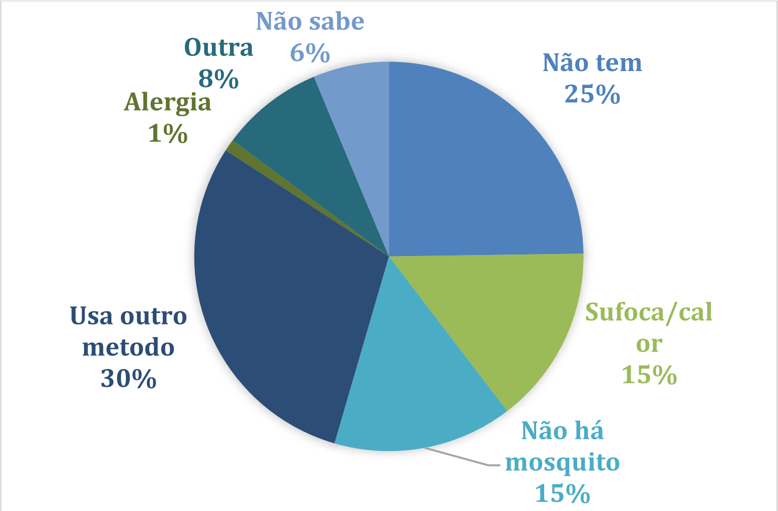

Figure 13. Reason for not using MII.

A logistic regression analysis was performed on a model including the following variables: region, area of residence, socioeconomic status, gender and age group to control for possible confounding factors for the use of MII. The regions of Biombo and Cacheu, the urban area and the last quintile of the socioeconomic level used the MII less ($p<0.00$) (Table 8).

Table 8. Driver-weighted use of MII, 2023.

Determinant	OR (95% CI)	p-value
Region:		
Bafatá	1.26 (0.86-1.84)	0.23
Bijagós	0.83 (0.48-1.43)	0.49
Screen	0.61 (0.42-0.89)	0.01
Bolama	0.64 (0.39-1.05)	0.08
Cacheu	0.61 (0.44-0.85)	0.04
Farim	2.19 (1.45-3.31)	<0.00
Gabú	1.27 (0.73-2.23)	0.40
Oio	1.30 (0.87-1.95)	0.19
Quinara	1.35 (0.86-2.11)	0.19
SAT	1	-
Tombali	0.87 (0.52-1.44)	0.58
Zone:		
Rural	1	-
Urban	0.72 (0.57-0.91)	0.00
Socioeconomic index:		
1st quintile	1	-
2nd quintile	1.11 (0.68-1.81)	0.68
3rd quintile	1.03 (0.77-1.38)	0.84
4th quintile	1.00 (0.72-1.40)	0.98
5th quintile	0.57 (0.39-0.81)	0.00
Sex:		
Female	1	-
Male	1.04 (0.94-1.15)	0.49
Age group:		
0 - 4	1	-
5 - 9	1.12 (0.93-1.34)	0.23
10 - 14	1.22 (1.02-1.46)	0.03
15 – 24	1.00 (0.84-1.19)	0.99
25 – 49	0.81 (0.70-0.94)	<0.01
>=50	0.63 (0.52-0.77)	<0.00

5.4. Evolution of the indicators of availability and use of MII and comparison with other African countries

Ownership of MII has increased considerably from 47% in 2010 to 97% of households that own at least one MII since 2011 with the three-year mass distribution has remained high. Ensuring universal access by providing at least one IIM for every two people in households is lower, currently standing at 77% (all mosquito nets impregnated) (Figure 14).

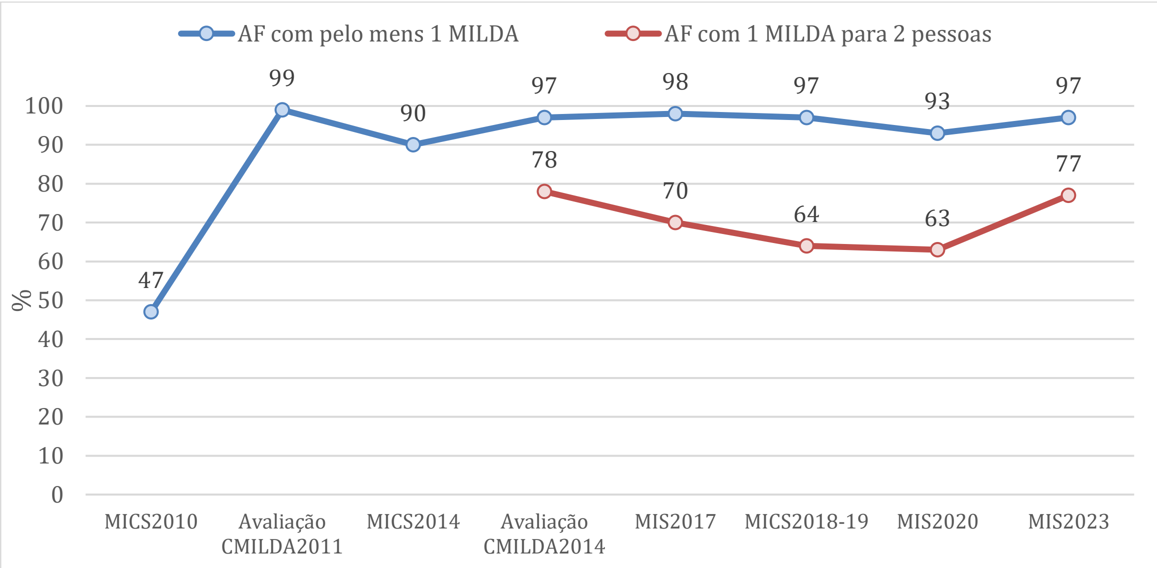

Figure 14. MII availability trend 2010-2023.

The use of IIMs has also increased considerably since 2011 in all target groups, and now stands at 87% in the general population (Figure 15).

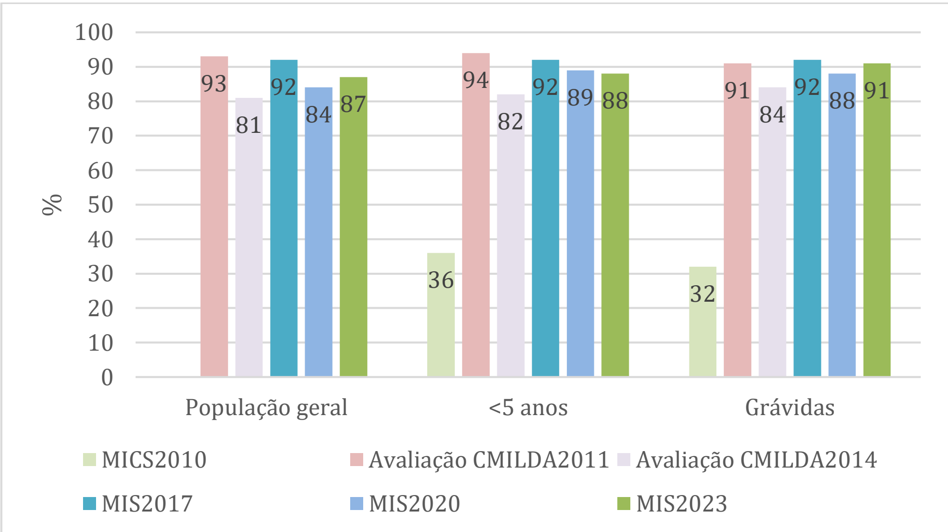

Figure 15. Trend of MII usage over the years 2010-2023.

In fact, the possession and availability of MII in households in Guinea-Bissau has been quite high compared to other West African countries, such as Senegal, The Gambia or Ghana, as well as other African regions, such as Mozambique, using data from MIS surveys conducted in these countries (Figure 16).

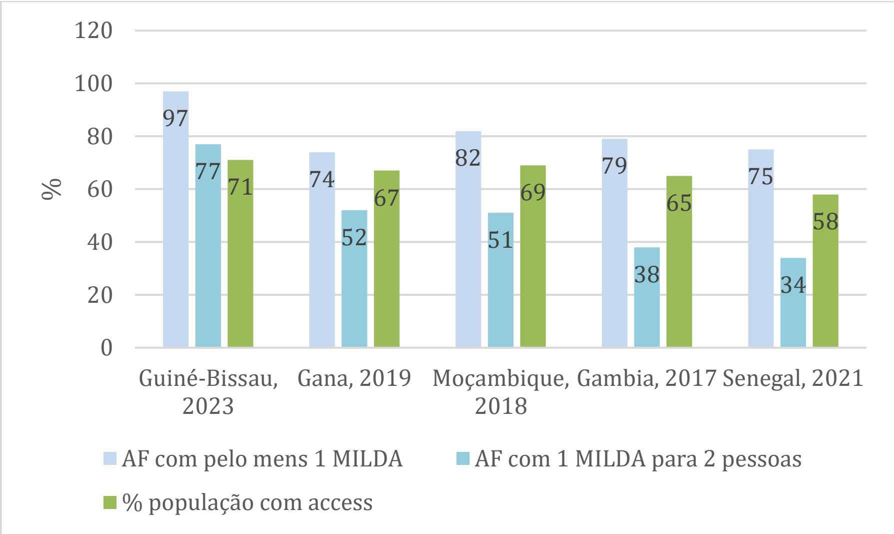

Figure 16. Possession and availability of MII in some African countries in MIS surveys.

In Guinea-Bissau, the use of an insecticide-impregnated bed net is comparatively higher than in different African countries, including Senegal, for which in the specific groups of children < 5 years and pregnant women the use is higher (Figure 17).

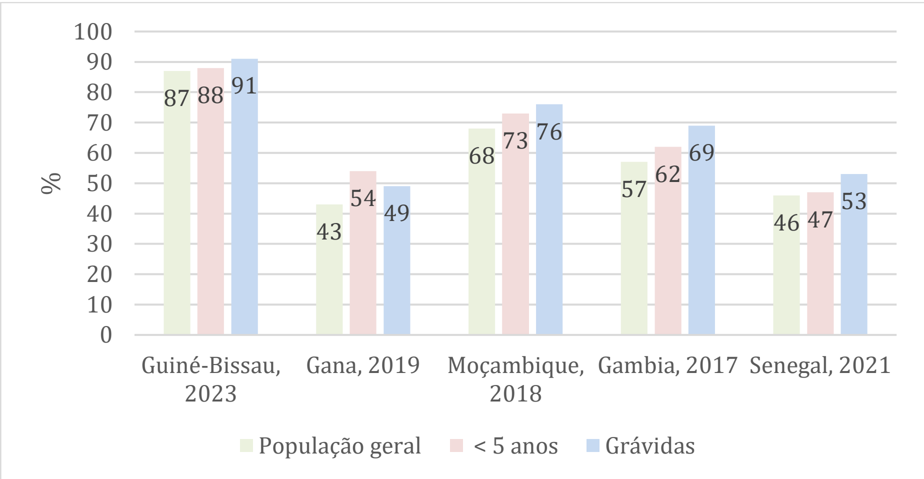

Figure 17. Use of MII in some African countries in MIS surveys.

5.5. Exposure to malaria messages in the past six months and information about MII

The questions about knowledge of MII were applied to only one main respondent from each household, without the help of the other members of the FA. A total of 4,821 individuals were interviewed, of which 3,051 (67%; CI: 65-69) reported having heard a message about malaria in the last six months, with very few (29%) in Farim having heard any message (Figure 18).

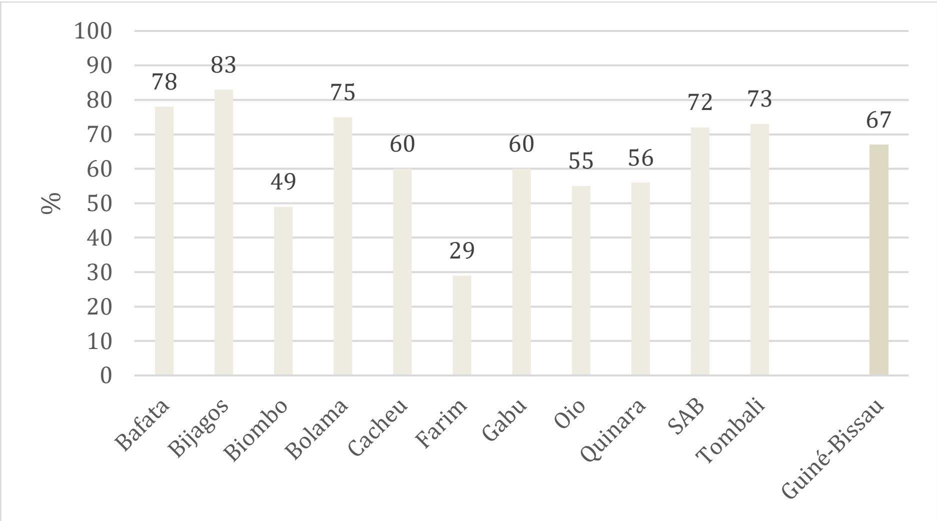

Figure 18. Exposure to malaria messaging in the past six months.

Respondents from urban areas had greater exposure to messages (70%; CI: 66-73) than in rural areas (64%; CI: 60-67) ($p=0.02$), as well as men (71%; CI: 68-74) compared to women (64%; CI: 62-67) ($p<0.00$) were more exposed to malaria messages in the last six months.

The most frequent source from which they heard messages about malaria in the last six months was radio (68%), followed by television (29%), health workers (28%), community health workers or community activists (23%) and SMS (16%) (Figure 19).

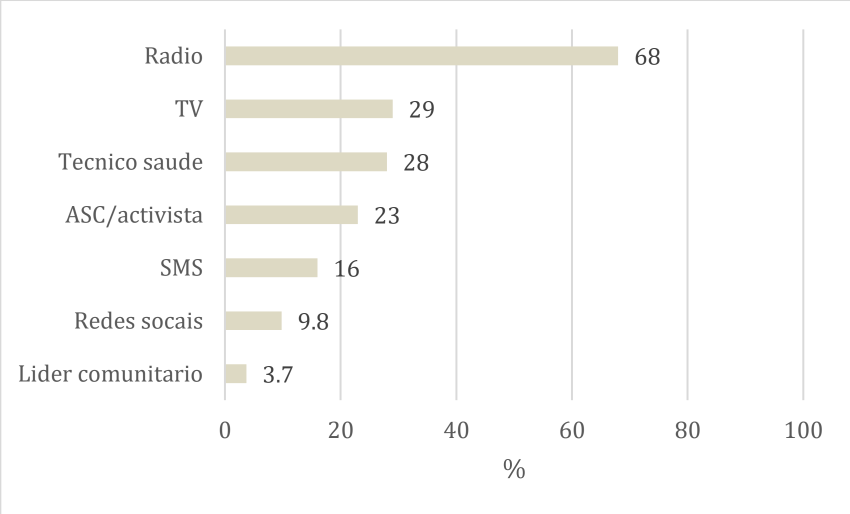

Figure 19. Source of malaria messages in the last six months.

About 94% (CI: 92-95) of households reported having received MII during the CMILDA2023, noting that the regions of SAB (91%), Cacheu (93%) and Biombo (94%) were those with the least PA that reported having received it (Figure 20).

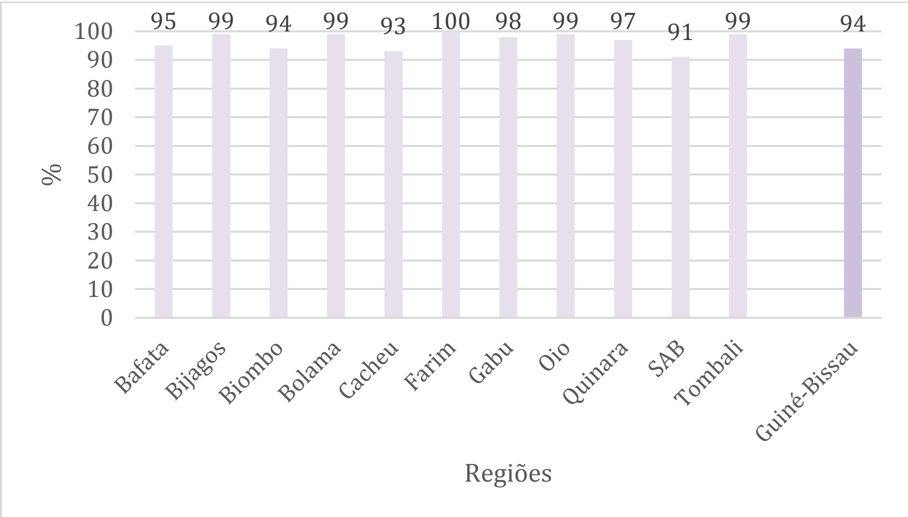

Figure 20. Declaration of receipt of MII in CMII2023.

According to the respondents, the main reasons why these AFs did not receive MII during the 2023 campaign were: household was not registered and did not receive a password (35%) and absence on the day of distribution (39%) (Figure 21).

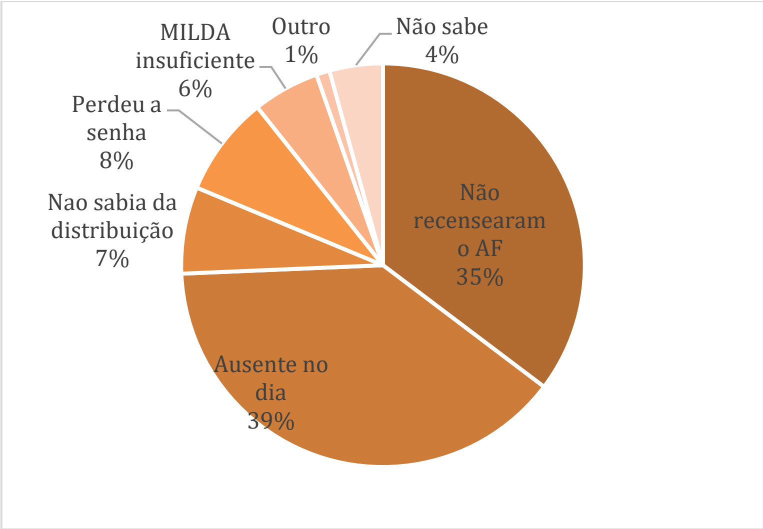

Figure 21. Reason for the household not receiving MII during CMII2023.

5.6. Intermittent preventive treatment in pregnancy for malaria prevention

A total of 1,841 women who had given birth in the last 24 months were registered, of whom 1214 (66%) had a prenatal consultation card and 98% (CI: 97-99) had had at least one antenatal consultation. The mean age of these women was 27 years; 64% of the women had attended school.

Coverage of intermittent preventive treatment for malaria prevention in pregnant women using sulfadoxine-pyrimethamine among other indicators was assessed in a total of 1,788 women.

The proportion of pregnant women who had at least one prenatal visit with ICT1 was 95% (CI: 9497) and the proportion of IPR3 was 54% (CI: 51-57), with differences between the regions (Figure 22).

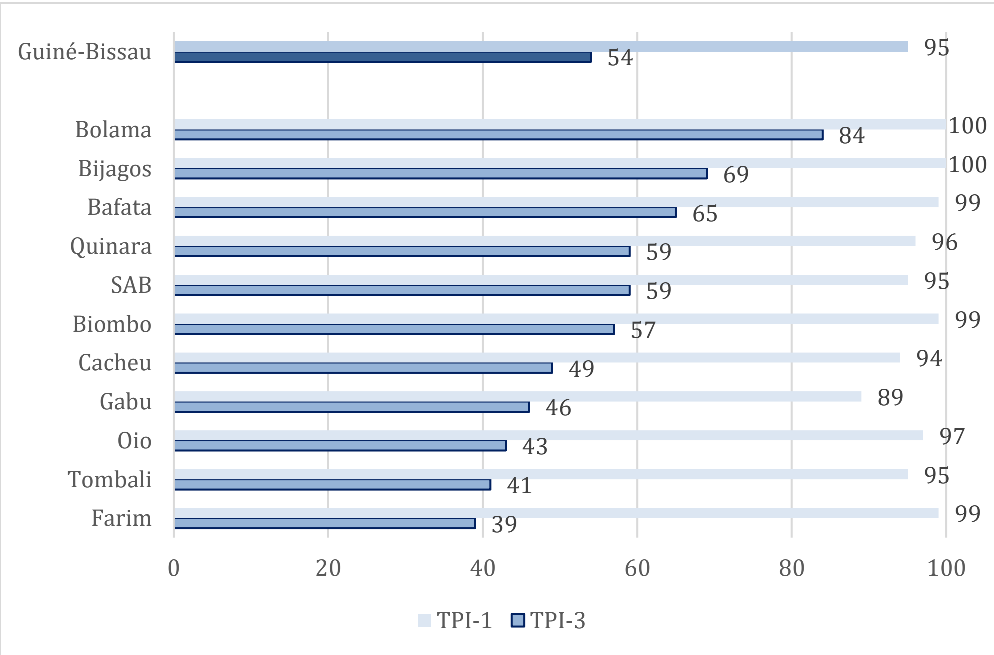

Figure 22. Proportion of pregnant women with NCC1 who took TPI1 and TPI3.

ICT3 coverage remains low and has not reached the target, although an increase has been observed over the years (Figure 23).

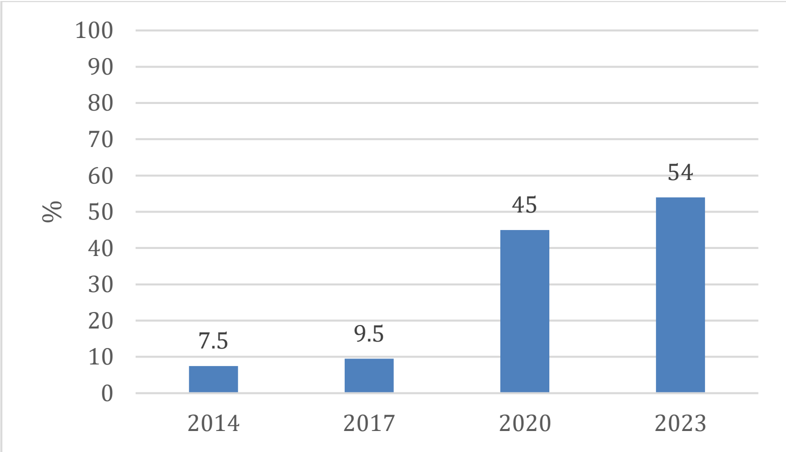

Figure 23. Evolution of ICT3 coverage from 2014 to 2023.

The main reasons why pregnant women did not take at least three doses of TPI were because they did not have enough antenatal visits (40%), simply because the technician did not give them (28%) and 15% did not know if they should take all three doses (Figure 24).

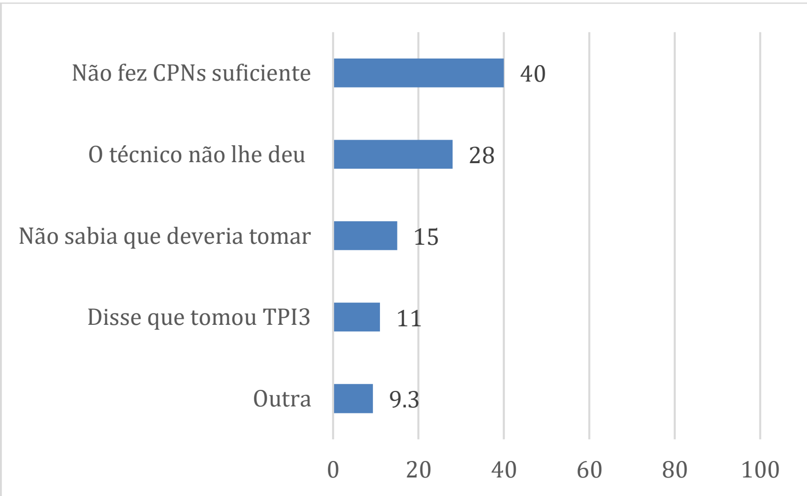

Figure 24. Evolution of ICT3 coverage from 2014 to 2023.

The proportion of assisted deliveries in health facilities was also evaluated, which was 71% (CI: 67-74). As expected, in the capital Bissau the proportion was quite high at 93% (Figure 25).

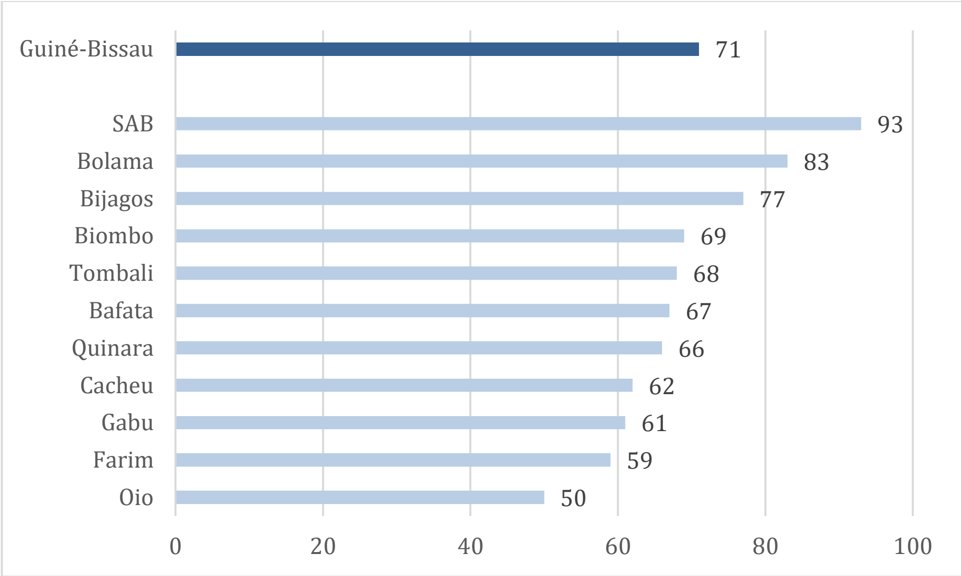

Figure 25. Assisted births in health facilities, by region.

5.7. Occurrence of fever in the community and seeking treatment

5.7.1. Fever in children under five years of age

Regarding the occurrence of fever in the last two weeks prior to the interview, including the day itself, of 4,826 children under five years of age included, 43% (CI:40-46) had fever (Figure 26).

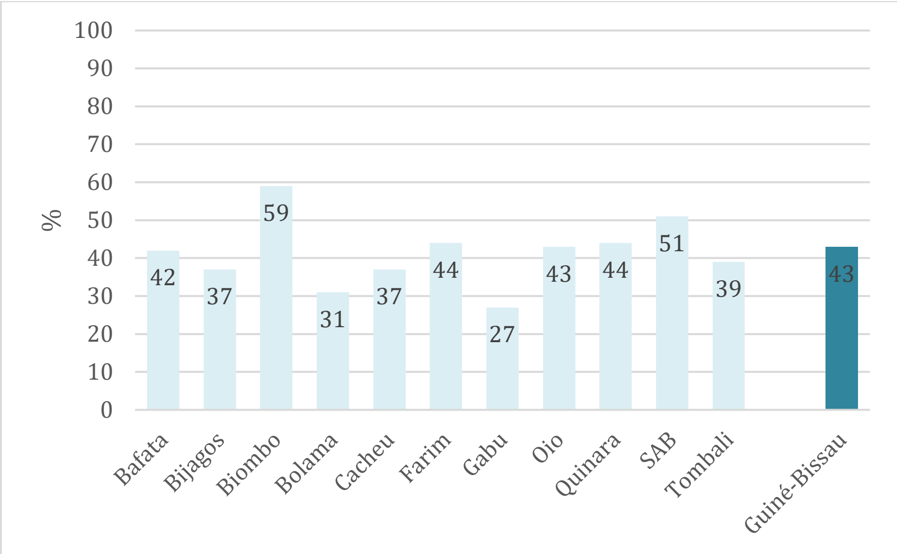

Figure 26. Occurrence of fever in the last two weeks, children <5 years old.

Approximately 38% (CI: 34-43%) of children who had had fever sought treatment in a public or private health facility. About 24% (CI: 21-29) reported having sought treatment within 24 hours of the onset of the disease (Figure 27).

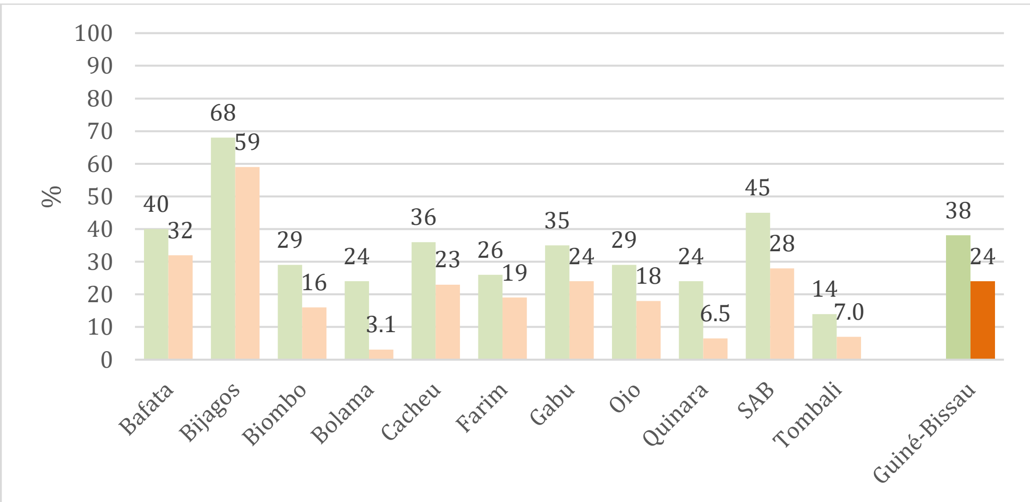

Figure 27. Place and time of seeking treatment, <5 years with fever in the last two weeks.

Of the children who sought help for treatment in general, 86% sought a public or private health facility, 3.7% sought a pharmacy, 6.5% a private clinic, 2.3% ASC and 1.2% a healer.

The proportion of children with fever in the past two weeks who had been bitten on the finger or heel (proxy for malaria testing) was 57% (CI: 50-64). For those with fever and who sought a health facility, the proportion of bites was 60% (CI: 53-66).

5.8. Seasonal malaria chemoprophylaxis in children from three to 59 months of age

Seasonal chemoprophylaxis for malaria prevention was administered to children aged 3-59 months in the regions of Bafatá, Gabú, Tombali, and Bolama from August to December 2023. As the survey was carried out in October-November, only the first two rounds were evaluated. Of the 2013 children aged 4 to 59 months included, the coverage of QPS-2, i.e., those who received prevention in at least two rounds, was 86% (CI: 81-90) and QPS-1 was 93% (CI: 91-94) (Figure 28).

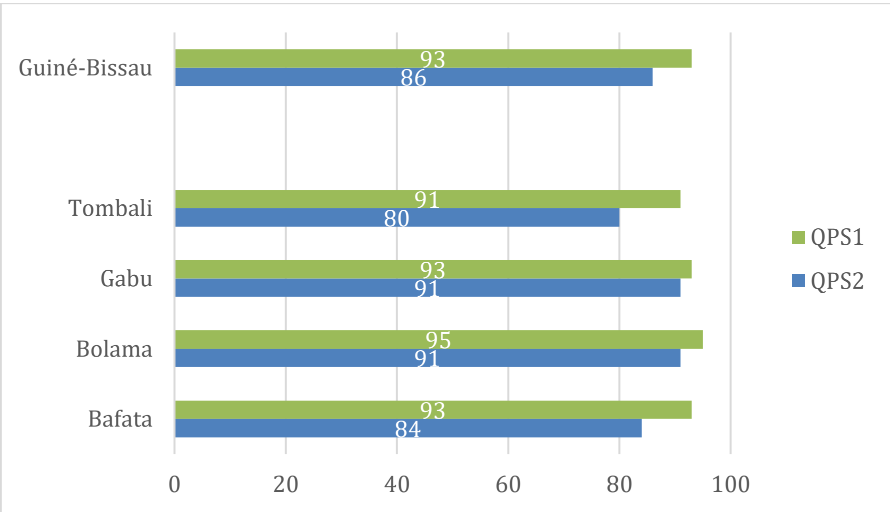

Figure 28. Coverage of one and two rounds of QPS in the regions in 2023.

Approximately 17% of the family members reported that there were adverse effects after taking amodiaquine/sulfadoxine-pyrimethamine (44%) and diarrhea (22%).

The main reasons given for non-participation in the QPS were because the child was absent (27%), the mother or guardian was absent (24%) and the team did not register them.

5.9. Prevalence of malaria infection in the community

5.9.1. Sample Description

For the study of the prevalence of *Plasmodium* in the community, 10,756 people present at the time of the home visit were asked for consent, of which 20 (0.2%) refused to do the interview. Of the 10,736 interviewees, 319 (3.0%) did not have laboratory results due to refusal or other reasons, remaining (Table 9).

Table 9. Screening and participation in the prevalence study.

Region	Total	Refusal of the interview	TDR Refusal	No information about testing	Undetermined result	Total with TDR and result
Bafatá	1 577	0	3	13	2	1 599
Bijagós	408	4	5	9	0	390
Screen	752	0	50	13	0	689
Bolama	320	2	3	6	0	309
Cacheu	1 302	2	9	11	1	1 279
Farim	571	2	0	3	0	566
Gabú	1 739	0	16	18	0	1 705
Oio	1 201	2	12	37	1	1 149
Quinara	879	3	3	7	0	866
SAT	1 235	5	35	42	0	1 153
Tombali	772	0	16	4	0	752
Total	**10 756**	**20**	**152**	**163**	**4**	**10 417**

Of the 10,417 with RDT results, 68% were from rural areas and 60% were female. Children aged six months to five years represent 41%, the 5-9 age group 9.6%, the 10-14 age group 7.1%, the 15-24 age group 14%, the 25-49 year age group 20% and the 50-year-old age group 8.2% (Table 10).

With regard to schooling, 53% in general had attended school and 50% of women; The median of the finished class was 6th (interquartile range: 4-9).

Table 10. Characteristics of those included in the prevalence study.

Region	Total	Zone		Sex		Age group (years)					
		Rural	Urban	Male	Female						
						< 4	5-9	10-14	15-24	25-44	>=45
Bafatá	1 559	1 240	319	656	903	714	137	112	171	306	119
Bijagos	390	259	131	163	227	115	47	40	56	87	45
Screen	689	467	222	264	425	281	66	59	87	139	57
Bolama	309	254	55	120	189	118	24	19	45	69	34
Cacheu	1 279	907	372	539	740	512	113	92	168	270	124
Farim	566	501	65	209	357	306	58	25	61	88	28
Gabú	1 705	1 385	320	686	1019	777	185	97	230	291	125
Oio	1 149	880	269	448	701	494	107	79	155	231	83
Quinara	866	685	181	331	535	333	82	66	129	176	80
SAT	1 153	0	1 153	396	757	349	105	101	223	279	96
Tombali	752	564	188	313	439	301	71	48	111	157	64
Total	**10 417**	**7 142**	**3 275**	**4 125**	**6 292**	**4 300**	**995**	**738**	**1 436**	**2 093**	**855**

5.9.2. Prevalence of RDT malaria infection by geographic area

Of the 10,417 individuals six months of age and older, 412 tested positive on the RDT test. The overall prevalence including all ages was 3.1% (95%CI: 2.6-3.8). The regions with the highest prevalence were Gabú (12%; CI: 9.2-15), followed by Bafatá (5.1% (CI: 3.6-7.4) and Tombali (3.0; CI: 1.9-4.6) (Figure 29 and Table 11 in Appendix 2).

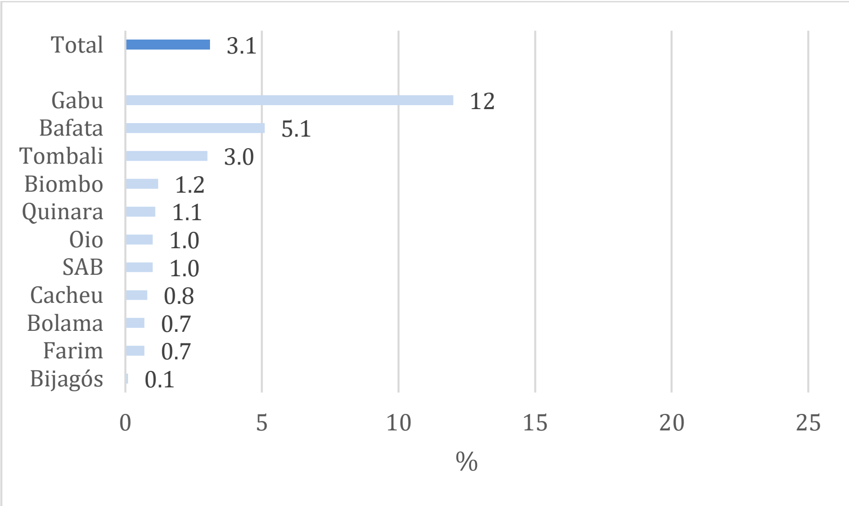

Figure 29. Prevalence of malaria infection, ≥ 6 months of age.

4,300 children aged between 6 and 59 months were tested, of whom 73 tested positive. The adjusted prevalence in this age group was 1.6% (CI: 1.1-2.2) globally, being 5.1% in Gabú and 1.9% in Bafata (Figure 30).

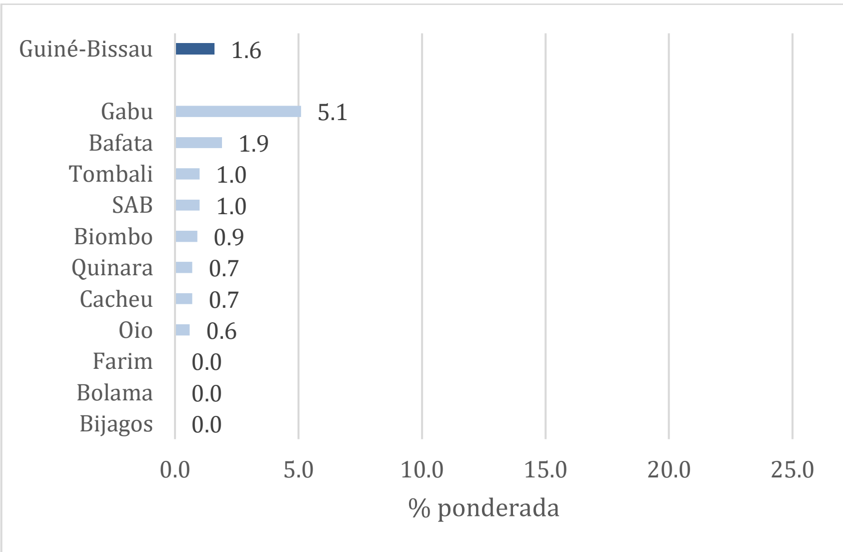

Figure 30. Prevalence of malaria infection in children aged 6-59 months.

Of the 6,117 individuals aged five years and over, 339 tested positive. The adjusted prevalence was 5.0% (CI: 4.1-6.1). For this age group, the prevalence was higher in the same regions of Gabú (19%), Bafatá (9.3%) and Tombali (6.0%) (Figure 31).

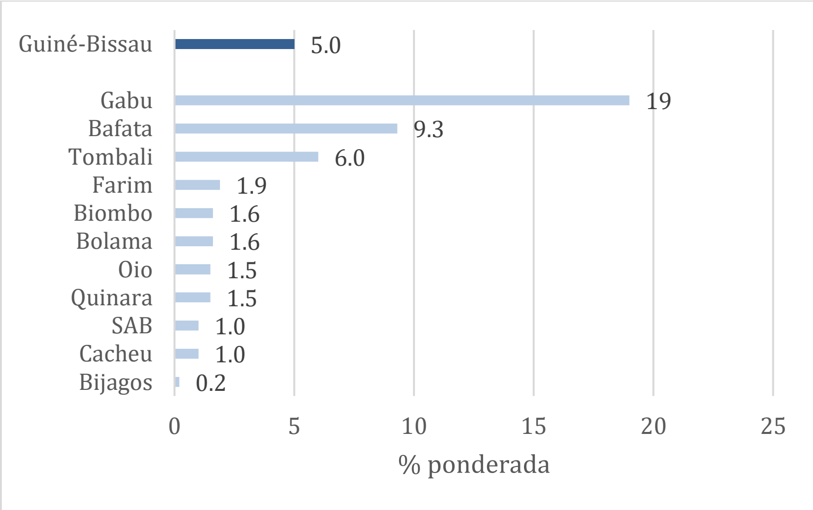

Figure 31. Prevalence of malaria infection, ≥ 5 years of age.

The overall prevalence in rural areas (4.5%; CI: 3.6-5.7) was significantly higher than in urban schools (1.7; CI: 1.1-2.4) ($p<0.00$).

Analyzing the geographical distribution of malaria infection at the regional level, it was observed that the eastern and southeastern areas of the country. However, analyzing by sector, the most affected areas were noted within the regions. In the Gabú region, the prevalence in children under five years of age was higher in Boé, Sonado and Gabú, while for those over 5 years of age it was in Sonaco, Pitche and Gabú. In the Bafatá region, the Cossé and Contuboel sectors showed a trend of higher prevalence; in Tombali, they were the Komo and Cacine sectors (Figures 32 and 33).

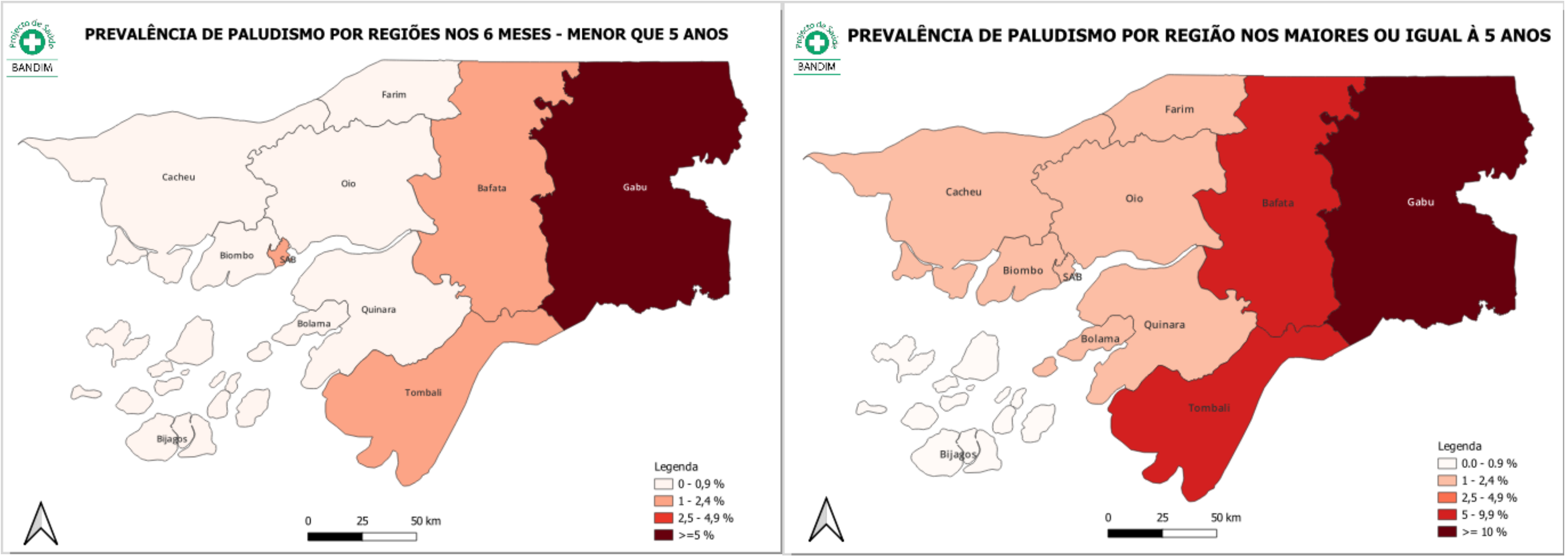

Figure 32. Distribution of malaria infection prevalence by region.

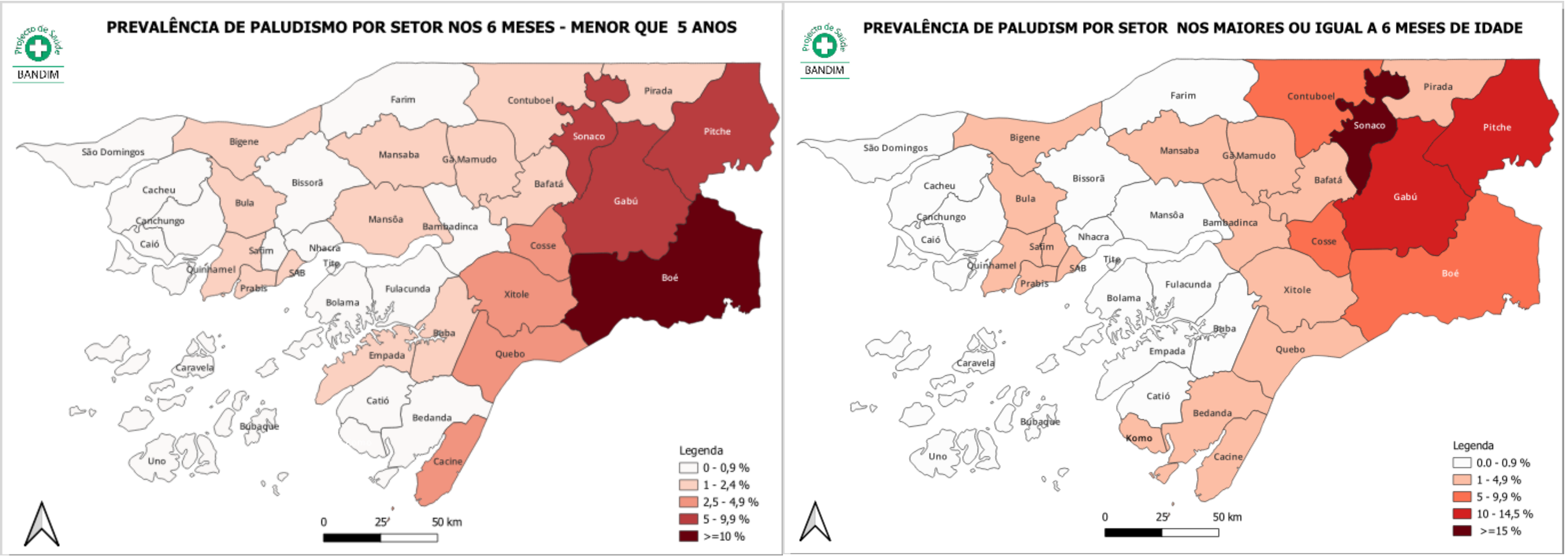

Figure 33. Distribution of malaria infection prevalence by sector.

5.9.3. Prevalence of RDT malaria infection by sex and age group

There was no difference in malaria prevalence in relation to gender, both for children under five years of age (1.6% in males versus 1.5% in females; p=0.90) and for those over 5 years of age (6.0% in males and 4.5% in females; p=0.09).

The prevalence was lower in children under five years of age, increasing and peaking in the age group of 5 to 10 years (7.9%; CI: 5.8-11) after which it declined with the increase in the age group (Figure 34 and Table 11 in Appendix 2).

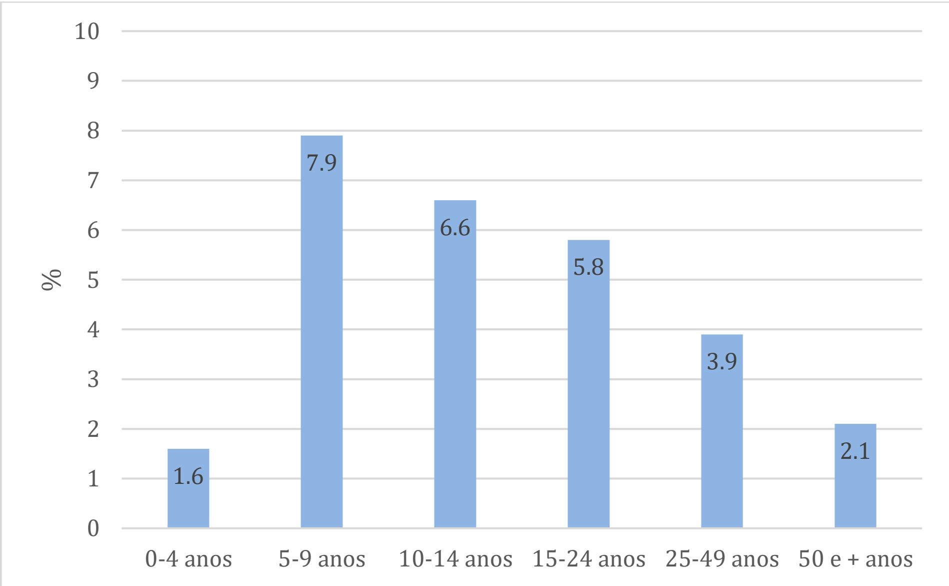

Figure 34. Prevalence of malaria infection stratified by age groups.

WHO recommends two malaria vaccines RTS,S/AS01 (RTS,S) and R21/Matrix-M in children in countries with moderate to high transmission of *P. falciparum*. The vaccine can be administered from 5 months of age in four doses (WHO 2024). Guinea-Bissau is considering submitting a proposal for funding application for the introduction of the vaccine, so it is important to include prevalence analysis stratified by ages below 59 months from now on.

The prevalence of malaria tended to increase from the age of two years (Table 12).

Table 12. Malaria prevalence stratified by age groups < 59 months.

Age group and months	TDR+/Tested	% (95% CI)
6-11	1/609	0.6 (0.1-4.2)
12-23	5/878	0.4 (0.2-1.1)
24-35	19/943	1.2 (0.7-2.2)
36-47	24/1011	2.4 (1.3-4.3)
48-59	24/859	2.9 (1.6-5.2)

5.9.4. Determinants of the prevalence of RDT infection

In addition to the factors described above, the association of malaria prevalence with other factors was assessed. Regarding socioeconomic status, only individuals belonging to the 5th level had a lower prevalence (1.3% against more than 3.5% in the other lower brackets). Schooling was also strongly associated with lower risk of infection (1.8% versus 5.4%; p<0.00) (Table 13).

Table 13. Prevalence of malaria infection due to other factors.

Determinant	TDR+/Tested	% (95% CI)	p-value
Socioeconomic level:			
First	82/1802	4.4 (3.0-6.3)	1
2nd	91/1963	3.8 (2.8-5.1)	0.47
Third	112/2300	4.5 (3.4-5.9)	0.09
4th	78/2330	3.5 (2.5-4.8)	0.33
5th	48/1900	1.3 (0.8-2.1)	<0.00
Schooling:			
No	288/4908	5.4 (4.3-6.7)	1
Yes	123/5434	1.8 (1.4-2.5)	<0.00
MII Usage:			
No	33/1053	2.3 (1.4-3.6)	1
Yes	375/10350	3.3 (2.7-3.9)	0.13
Had fever at 2 weeks:			
No	246/6682	3.0 (2.3-3.8)	1
Yes	166/3668	3.5 (2.7-4.5)	0.31
Took antimalarial:			
No	317/8601	2.8 (2.3-3.5)	1
Yes	90/1729	4.7 (3.4-6.3)	<0.01
Travel in the 2 weeks:			
No	378/9394	3.2 (2.6-3.8)	1
Yes	34/1017	3.2 (2.1-4.8)	0.98

Having had a fever in the last two weeks, including the day of inclusion, was not associated with a higher risk of infection, having already taken antimalarial drugs in the last two weeks, yes ($p<0.01$). However, the analysis with interaction between fever in the last two weeks and having taken antimalarial drugs was associated with twice as much positivity for RDT drugs ($p<0.00$), while only having had fever or having taken antimalarial drugs separately did not make any difference (Table 13).

In the logistic regression model including region, area of residence, socioeconomic status, age group, sex, education, use of MII, interaction of fever and antimalarial drug use, the factors independently associated with the highest prevalence were the regions of Gabú (OR=8.16; 3.81-17.5; $p<0.00$) with 8 times higher prevalence and Bafatá (OR=3.30; 1.42-7.67; $p<0.01$) with three times more than Bissau, age groups over five years old ($p<0.00$).

Having had fever and not taking antimalarial drugs in the last two weeks (OR=1.51; 1.02-2.23; $p=0.04$) and especially having had fever and taking antimalarial drugs (OR=2.50; 1.48-4.22; $p<0.01$) were associated with a higher prevalence of malaria infection. On the other hand, education OR=0.67; 0.48-0.94; $p=0.02$) was associated with lower prevalence (Table 14).

Table 14. Determinants of malaria infection prevalence in logistic regression.

Determinant	Odds ratio (95% CI)	p-value
Region:		
Bafatá	3.30 (1.42-7.7)	<0.01
Bijagós	0.08 (0.01-0.70)	0.02
Screen	0.87 (0.33-2.24)	0.76
Bolama	0.54 (0.17-1.73)	0.30
Cacheu	0.63 (0.24-1.69)	0.36
Farim	0.34 (0.09-1.33)	0.12
Gabú	8.16 (3.81-17.5)	<0.00
Oio	0.54 (0.20-1.46)	0.22
Quinara	0.67 (0.25-1.83)	0.44
SAT	1	-
Tombali	2.14 (0.89-5.15)	0.09
Urban vs rural area:	0.94 (0.64-1.38)	0.76
Socioeconomic level:		
First	1	-
2nd	0.86 (0.54-1.37)	0.52
Third	1.11 (0.67-1.84)	0.67
4th	1.10 (0.63-1.75)	0.85
5th	0.62 (0.31-1.24)	0.17
Male vs Female Gender:	0.81 (0.56-1.82)	0.27
Age group:		
6 months to 4 years	1	-
5 to 9 years	6.11 (3.34-11.2)	<0.00
10 to 14 years old	6.10 (3.13-11.9)	<0.00
15 to 24 years old	5.65 (3.02-10.6)	<0.00
25 to 44 years old	3.20 (1.87-5.48)	<0.00
45 +	1.31 (0.61-2.84)	0.49
Education- Yes vs No:	0.67 (0.51-0.94)	0.02
Fever + antimalarial:		
No and didn't take it	1	-
No and took	1.51 (0.43-1.47)	0.46

Yes and didn't take	1.51 (0.02-2.23)	0.04
Yes and took	2.50 (1.48-4.22)	<0.01
MII Usage – Yes vs No:	0.84 (0.51-1.39)	0.51

The quantitative polymerase chain reaction performed on a large number of samples needs sufficient time. For this reason and the importance of this result, this component will be subject to a separate report and publication ensuring all the necessary scientific requirements. Thus, collaboration with the Medical Research Council will continue in this field and in the creation of appropriate national capacities.

qPCR was performed on 10 195 DBS samples, and the test needed to be repeated on 2748 samples, in addition to quality control on a random sample.

Given the high number of samples to be analyzed and the possibility of contamination of the surface and equipment with genomic DNA, it was added to each test board, the duplicate analysis of the negative control (where water is used as a *template* instead of DNA) and *the Non template control* (NTC), i.e., composed only of mixing the reaction without the addition of DNA as a way to ensure quality control of VarATS assays. The presence of Ct value in the NTC well will indicate that there was contamination at the time of preparation of the PCR mixture and consequently the entire plate will be contaminated. The presence of a Ct value in the negative control may indicate that the sterile water used as a *template* is contaminated or the negative control was contaminated in the process of adding the positive control.

Thus, it was decided to reanalyze all plates in which both CNT/negative controls showed positive results in one or both replicates, and the samples analyzed in these plates should be subsequently reanalyzed in order to ensure a reliable diagnosis.

5.9.5. Evolution of malaria prevalence

Community-based malaria infection declined considerably from 2020 to 2023 across all age groups from 6.4% to 3.1%. In children under five years of age, there was a drop from 3.6% to 1.6%, in those over five years of age it was from 7.8% to 5.0% and, in the age group of 5-14 years of age, it decreased from 12% to 7.3% (Figure 35).

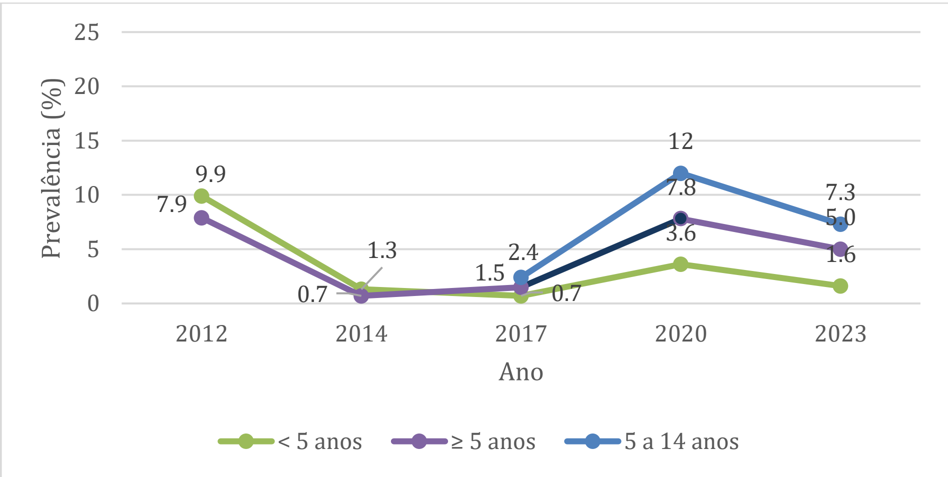

Figure 35. Trend in the prevalence of malaria infection in different age groups, 2012-2020.

In children under five years of age, a significant decline was observed in the regions with the highest prevalence in 2020, i.e., in Gabú, Bafatá, and Farim, but also in Quínara and Tombali (Figure 36).

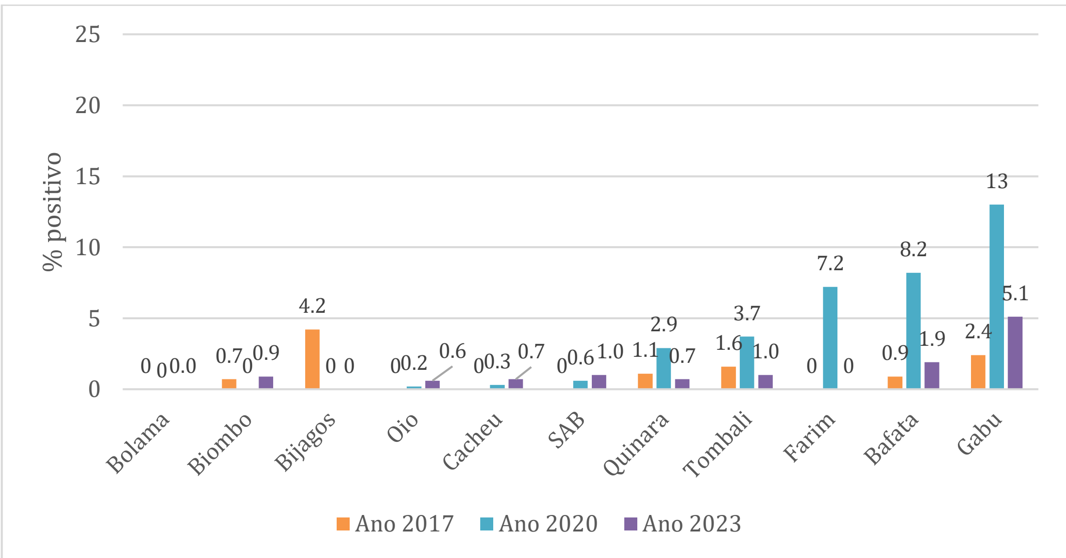

Figure 36. Evolution of malaria infection prevalence by region 2017-2023, 6-59 months.

In the age group of those over 5 years of age, a decrease in prevalence was also observed in most regions, even in the region of Gabú, which still has a high level of prevalence (Figure 37).

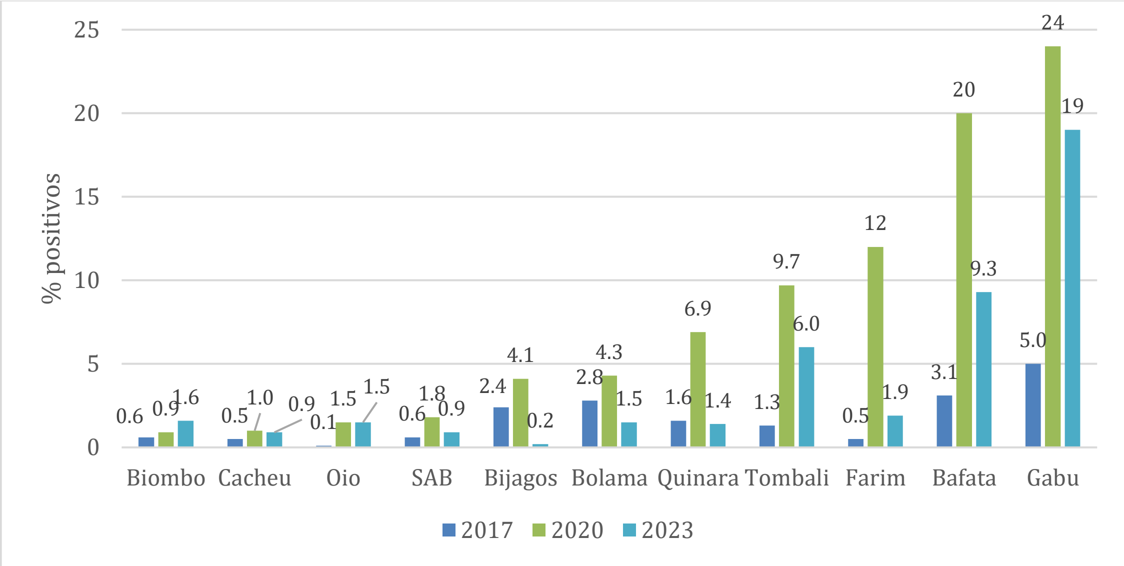

Figure 37. Evolution of malaria infection prevalence by region from 2017-2023, ≥ 5 years.

5.9.6. Prevalence of anaemia in children aged 6-59 months

The proportion of children aged 6-59 months with anemia, i.e., hemoglobin levels below 12 g/dL, was 68% overall (CI: 65-71), with some variation between regions (Table 15). There was a trend toward a lower prevalence of anemia in urban areas (65%; 60-69 versus 71; 67-74; p=0.06).

Table 15. Prevalence of anemia by region, 6-59 months of age.

Region	Hb < 12 g/dL/Tested	% (95% CI)
Bafatá	489/713	69 (64-74)
Bijagós	62/115	53 (41-64)
Screen	205/281	75 (69-80)
Bolama	102/116	88 (82-92)
Cacheu	343/512	60 (49-71)
Farim	267/306	87 (83-91)
Gabú	604/776	78 (74-82)
Oio	416/494	84 (79-87)
Quinara	235/333	73 (64-80)
SAT	227/349	63 (58-69)
Tombali	188/301	59 (51-67)
Total	**3 138/4 296**	**68 (65-71)**

6. CONCLUSIONS AND CONSIDERATIONS ON THE RESULTS

Key indicators of household availability of MIIs and their use remain high or acceptable in 2023. The proportion of households that have at least one MII was 97% and the proportion of PA with one MII for two people was 77%. In all target groups, the use of MII remains at more than 80% at: the proportion of the population that used MII the night before was 87%, of children under five years of age was 88% and of pregnant women 91%.

Most of the MII come from the CMILDA and most households are affected. It should be noted that about 20% use MII from CMILDA 2020, mainly due to the preference of the quality of these MIIs in terms of size, design with opening ("door") and softer material. Prevention of specific groups, such as intermittent preventive treatment of pregnant women with at least three doses, increased compared to 2020 and stands at 54% in 2023 (PSB 2020).

Community-based malaria infection declined considerably from 2020 to 2023 across all age groups, falling from 6.4% to 3.1% in the general population. In children aged 6-59 months, there was a drop from 3.6% to 1.6% and in individuals aged 5 years and over it fell from 7.8% to 5.0%. The decline was significant in the regions with the highest prevalence in 2020, which are Gabú, Bafatá and Farim, but also in Quínara and Tombali. However, in the region of Gabú and Bafatá, a high level of prevalence is still observed, especially in those over five years of age.

An independent association was observed between some risk factors and prevalence. Schooling (OR=0.67; 0.48-0.94; $p=0.02$) was the only factor associated with lower prevalence. The factors associated with higher prevalence were the region of Gabú (OR=8.16; 3.81-17.5; $p<0.00$) with 8 times higher and Bafatá (OR=3.30; 1.42-7.67; $p<0.01$) with three times higher prevalence than Bissau; Another important factor was the age at which the age groups over five years ($p<0.00$) had the highest prevalence.

Having had fever and not taking antimalarial drugs in the last two weeks (OR=1.51; 1.02-2.23; $p=0.04$) and especially having had fever and taking antimalarial drugs (OR=2.50; 1.48-4.22; $p<0.01$) were associated with a higher prevalence of malaria infection. This situation among those who took medication may have been because they did not complete the treatment, but also because the RDT continued to be positive a few weeks later, even if the patient no longer had the infection (Dalrymple *et al*. 2018; Hosch *et al*. 2022).

Another challenge opposed to the previous issue is the non-detection of infections with low parasite density by RDT and microscopy and the maintenance of possible foci of transmission, so the use of qPCR in this survey to know the situation, however this issue will be the subject of a separate report.

It is also worth mentioning the moderate to high resistance of *Anopheles* mosquito populations to pyrethroids, insecticides used in the country, in the bioassays carried out in 2018, as well as the high frequency of *kdr mutation* and the partial involvement of metabolic resistance and/or penetration reduction, as well as the use of MII with synergist PBO (piperonyl butoxide) in the regions of Gabú, Bafatá, Tombali and Bolama.

Progress has been observed in malaria control and every effort should be made to capitalise it towards elimination ambitions in the future. It is important to closely monitor the epidemiological situation and barriers in order to quickly identify bottlenecks, outlining specific interventions adapted to local specificities.

7. RECOMMENDATIONS

Based on the observations, the main recommendations to improve malaria control outcomes are as follows:

a) Assess and follow at a more stratified level the areas and groups that carry the greatest risk, as well as the local determinants, particularly in the regions of Gabú and Bafatá;

b) Define specific intervention strategies with the age groups of those over 5 years old on a pilot basis in the region of Gabú and Bafatá;

c) Assess the quality and impact of QPS campaigns in the regions of Gabú and Bafatá, as well as further assess the determinants of infection in these two regions.

d) To study in more detail the level of under-detection of RDT infection not only based on MIS 2023 samples, but also from clinical samples from sentinel posts.

e) Pay particular attention to the acquisition of MII with acceptable dimensions (that are larger than those of CMILDA2023), smooth material and, if possible, with openings. The quality of MIIs can increase utilization;

f) Maintain the use of MII with synergist PBO for the targeted regions in 2023 at least until further evidence.

g) Maintain active awareness and mobilization of the population for prevention and care measures.

8. REFERENCES

Dalrymple U, Arambepola R, Gething PW, Cameron E. How long do rapid diagnostic tests remain positive after anti-malarial treatment? Malar J 2018; 17: 228.

Del Ninno, Carlo and Bradford Mills, 2015 (Eds.), Safety Nets in Africa: Effective Mechanisms to Reach the Poor and Most Vulnerable. The World Bank, Washington D.C.

Filmer D, Pritchett L. Estimating Wealth Effects Without Expenditure Data – Or Tears: An Application to Educational Enrollments in States in India. Demography 2001; 38:1.

Ghana Statistical Service (GSS) and ICF. Ghana Malaria Indicator Survey 2019. Accra, Ghana and Rockville, Maryland, USA. GSS and ICF 2020.

Hosch S,Yoboue CADonfack OT Guirou ANDDangy JPMpina M, et al. Analysis of nucleic acids extracted from rapid diagnostic tests reveals a significant proportion of false positive test results associated with recent malaria treatment. Malar J 2022; 21: 23.

National Institute of Health (INS) and IFC: National Survey on Malaria Indicators in Mozambique 2018. Maputo, Mozambique. Rockville, Maryland, USA: INS and ICF 2019.

Instituto Nacional de Saúde Pública: Étude de la susceptibilité des vecteurs du paludisme aus insecticides en Guinee Bissau. Final rapport. INASA, 2017.

National Institute of Public Health: Evaluation of the impact of the long-term mosquito net distribution campaign in Guinea-Bissau. INASA, 2015.

National Institute of Public Health: Evaluation of the impact of the long-term mosquito net distribution campaign in Guinea-Bissau. INASA, 2012.

National Institute of Public Health: Guinea-Bissau Statistical Health Yearbook 2018. INASA, 2018.

Kenya Legal and Ethical Issues Network on HIV & AIDS (KELIN). Matchbox malaria in Guinea-Bissau: Study on malaria vulnerability, protection of rights, and barriers to access to health services. KELIN 2019.

Konate L. Renforcement des capacités et étude de la sensibilité aux pyrethrinoides des vecteurs de paludisme en Guinée Bissau. Final rapport. LEVP BA FST UCAD 2009.

Lalloué B, Monnez J, Padilla C, Kihal W, Le Meur N, Zmirou-Navier D, Deguen S. A Statistical Procedure to Create a Neighborhood Socioeconomic Index for Health Inequalities Analysis. International Journal for Equality in Health 2013; 12:21.

Mappin B, Cameron E, Dalrymple U, Weiss DJ, Bisanzio D, Bhatt S, et al. Standardizing Plasmodium falciparum infection prevalence measured via microscopy versus rapid diagnostic test. Malar J. 2015; 14:460.

MEASURE Evaluation, The Demographic and Health Surveys Program, President's Malaria Initiative, Roll Back Malaria Partnership, United Nations Children's Fund,

Ministry of Economy, Planning and Regional Integration - General Directorate of Planning. Multiple Indicators Survey, Reproductive Health Demographic Survey. Main results, preliminary report. MEPIR, Guinea-Bissau, 2014.

Ministry of Economy, Planning and Regional Integration - General Directorate of Planning. Multiple Indicators Survey, Reproductive Health Demographic Survey. Final Report. MEPIR, Guinea-Bissau, 2011.

Agence Nationale de la Statistique et de la Démographie (ANSD) [Sénégal], et ICF. 2021. Enquête sur les indicateurs du paludisme au Sénégal, 2020-2021. Rockville, Maryland, USA: ANSD et ICF.

Organization Mondial de la Santé. Guide pour l'évaluation de la mise en œuvre, des réussites et du rendement des campagnes de masse pour l'imprégnation des moustiquaires et autres matériaux imprégnés d'insecticides (CIM), dans les districts sélectionnés de la région Africaine. WHO 2002.

Poti K, Sullivan DJ, Dondorp AM, Woodrow CJ. HRP2: Transforming Malaria Diagnosis, but with Caveats. Trends in Parasitology 2020; 36: 112-26.

National Malaria Control Programme: Annual Report 2020. PNLP 2021.

Bandim Health Project: 2020 Malaria Indicators Survey in Guinea-Bissau - Report. PSB, 2020.

Roll Back Malaria Partnership. Global Malaria Action Plan. RBM, Geneva 2008. http://rbm.who.int/gmap/toc.html.

Rodrigues A, Schellenberg J, Kofoed PE, Aaby P, Greenwood B. The changing pattern of malaria in Bissau, Guinea-Bissau. Trop Med Int Health 2008; 13: 1-8.

Shepard DS, Odumah JU, Awola ST. Cost-effectiveness of PBO versus conventional long-lasting insecticidal bed nets in preventing symptomatic malaria in Nigeria: results of a pragmatic randomized trial. Am J Trop Med 2021; 104: 979-86.

Silva R, Mavridis K, Vontas J, Rodrigues A, Osório HC. Monitoring and molecular profiling of contemporary insecticide resistance status of malaria vectors in Guinea-Bissau. Acta Trop 2020; 206: 105440.

The Gambia National Malaria Control Programme, The Gambia Bureau of Statistics and Utica International. The Gambia Malaria Indicator Survey 2017. Columbia, Maryland, USA: The Gambia NMCP, The Gambia Bureau of Statistics and Utica International 2018.

UNDP: Human Development Report 2020. The Next Frontier : Human Development and the Anthropocene. UNDP 2020.

United Nations. The Millennium Development Goals Report 2014. UNDP, New York 2014.

Victora CG, Barros AJ, Axelson H, Bhutta ZA, Chopra M, France GV, Kerber K, Kirkwood BR, Newby H, Ronsmans C, Boerma JT. How changes in coverage affect equity in maternal and child health interventions in 35 Countdown to 2015 countries: an analysis of national surveys. Lancet 2012; 380:1149-56.

Vyas S, Kumaranayake L. How to do (or not to do). Constructing Socio-Economic Status Indices: How to Use Principal Components Analysis. Oxford University Press 2006.

World Health Assembly: Global Technical Strategy for malaria 2016-2030. World Health Organization, 2016.

Malaria Vaccines: WHO position paper. Weekly Epidemiological Record 2024; 19: 225-48.

World Health Organization and Global Malaria Programme. Global Fund concept note development. WHO Policy Brief. WHO 2014.

World Health Organization: Household Survey Indicators for Malaria Control. WHO, 2018.

Ye Y, Patton E, Kilian A,Dovey S, Eckert E. Can universal insecticide-treated net campaigns achieve equity in coverage and use? the case of northern Nigeria. Malar J.2012 Feb 1;11:32. doi: 10.1186/1475-2875-11-32.

9. TECHNICAL PROFILE

Project title:	National Survey on Malaria Indicators (MIS2023)
Drawing:	Quantitative cross-sectional survey
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Financing:	NFM3, Global Malaria Fund through UNDP Guinea-Bissau

10. List of Tables and Figures

List of Tables

List of figures

Figure 37. Evolution of malaria infection prevalence by region 2017-2023, ≥ 5 years

ATTACHMENTS

Annex 1. Diagnosis of Plasmodium falciparum infections in qPCR using the VarATS assay

A. Context

The standard methodologies used for the diagnosis of malaria in the country are light microscopy and rapid diagnostic tests based on the detection of histidine-rich protein 2 (PFHRP2) specific to the parasite *Plasmodium falciparum*. These methodologies are crucial in malaria control in endemic and low-income countries such as Guinea-Bissau, however, as reported in several studies, they have limitations and consequently miss the diagnosis of infections with low parasite densities. Given the reduction in the prevalence of malaria, asymptomatic and low-parasite density infections have emerged that are not detectable by standard diagnostic methodologies and consequently untreated, contributing as silent reservoirs for malaria transmission.

In this sense, it is necessary to use more sensitive molecular methodologies, especially in surveillance studies in order to have a more comprehensive and realistic view of the prevalence and dynamics of malaria epidemiology in different contexts.

Blood samples on filter paper collected during MIS 2023 were analysed using molecular methods, targeting a sequence of the parasite's genome composed of multiple copies, which allowed for greater sensitivity in detecting low-density infections. The method used was described in the article by (Hofmann et al., 2015) and consists of the use of quantitative polymerase chain reaction (qPCR) for ultra-sensitive detection of *Plasmodium falciparum*, by amplifying a conserved segment of the ATS region present in all genes of the gene family *Var* (varATS), that is, composed of about 59 copies/genome. This technique allows the detection of 0.03 to 0.15 parasites/µl of blood and is 10× more sensitive than the standard 18S rRNA qPCR PCR.

B. Procedures

a. DNA extraction

Numerical values were assigned to each sample on filter paper collected during the MIS2023. 3 circles of 6mm diameter of paper containing blood were cut and placed in individual *eppendorfs* tubes duly identified with the assigned numbers.

The cutting and preparation of the filter paper samples was carried out at the National Public Health Laboratory (LNSP). The technicians were trained following the work protocol provided by the consultant (attached).

The samples stored in individual *eppendorfs* tubes were then sent to Jean Piaget University for DNA extractions using the Biobase Automatic Nucleic Acid Extraction System.

The extracted DNA was sent daily to the LNSP in DNA extraction plates where they were kept as stock for later use if necessary quality control.

At LNSP, working plates composed of 20 µl of DNA extracted in pre-prepared plates were prepared according to the qPCR reaction plate. The DNA-containing plates were stored at -80ºC.

b. Quantitative PCR (qPCR-VarATS)

The PCR reaction was prepared and analyzed following the protocol described in the literature (Hofmann et al., 2015) and adapted by a technical assistant from The Medical Research Council Unit, The Gambia.

The Bio-Rad CFX Maestro device available at the National Public Health Laboratory (LNSP) was used.

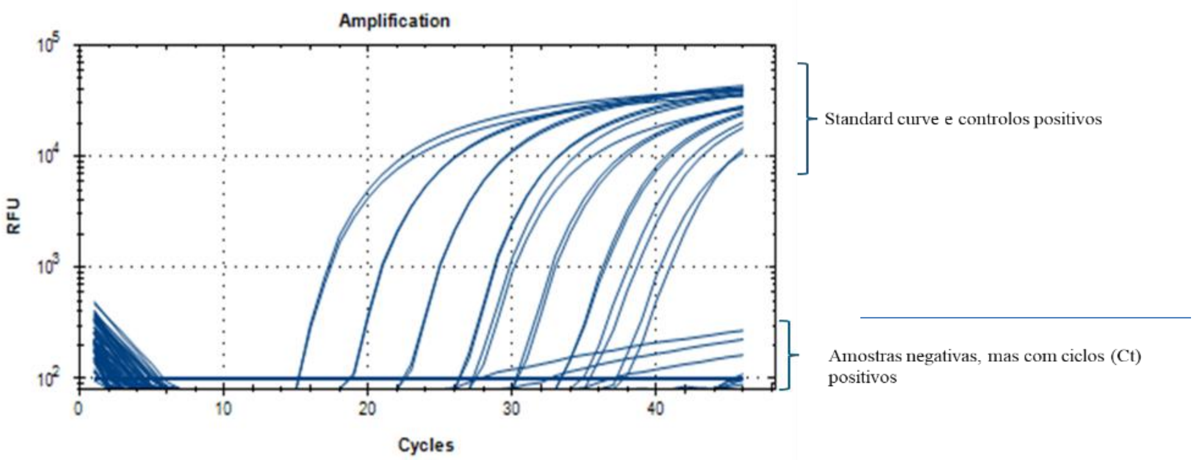
A serial dilution of DNA samples with known parasite density was used to estimate the parasite density of the field isolates (MIS2023 samples).

Each qPCR plate was composed of a standard curve, i.e., serial dilution analyzed in duplicate and 74 samples of field isolates, two negative controls (using water instead of the genetic material), two positive controls (DNA) and two wells with only the reaction mixture (NTC).

	1	2	3	4	5	6	7	8	9	10	11	12
A												
B												
C												CN
D	St. Curve	St. Curve										CP
E												CN
F												CP
G												NTC
H												NTC

Field isolates

The ideal recommended parameters for the analysis of the results were an efficiency of 80%-110%, a *slope* of 3.4 and an R2 of 0.99. The *treshold* was set manually to 100. Positive samples were those with a Ct value ≥14.1-≤35.9, negative samples were considered to be all samples with a Ct lower than 14 and ≥ 39.1. Samples with Ct 36-≤39 were considered ultra low density. Samples whose Ct values were considered positive, but with a standard curve different from the Standard curve, were considered negative for P*. falciparum infection.*



Comments and recommendations for the future

The following challenges were encountered during the trials:

- Due to the error in the description of the primers (Hofmann et al., 2015) that was not corrected during publication, there was a need to make an extra purchase of *Primers* appropriate to the test;
- A high number of samples with Ct considered positive, but with an indicative curve of nonspecific amplification, possibly due to the high amount of DNA extracted by the manual extraction equipment, was observed;
- The limitation and dependence on the supply of the standard curve led to delays throughout the performance of the tests. It was later filled by diluting the DNA corresponding to the first concentration of DNA.

The following recommendations are given for general improvement of future work:

- Creation of conditions for separating the clean area from the dirty area at LNSP;
- Need for stock of PCR reagents and disposables for use to confirm the operation of the most sensitive assays;
- Creation of a national standard curve library;
- Repeat analysis of the plates excluded due to the presence of negative control and contaminated NTC.

Annex 2. Malaria prevalence

Table 11. Prevalence of malaria infection in the age group 6 to 59 months and 5 years and over by region.

Region	Age group 6 months-4 years		Age group 5 years and over	
	TDR+/Tested	% (95% CI)	TDR+/Tested	% (95% CI)
Bafatá	14/714	1.9 (1.0-3.6)	83/845	9.3 (6.1-14)
Bijagós	0/115	0	1/275	0.2 (0.0-1.5)
Screen	3/281	0.9 (0.3-2.7)	6/408	1.6 (0.9-3.0)

Bolama	0/118	0	3/191	1.6 (0.7-3.6)
Cacheu	5/512	0.7 (0.2-1.8)	8/767	1.0 (0.5-2.1)
Farim	0/306	0	5/260	1.9 (0.8-2.1)
Gabú	39/777	5.1 (3.2-8.0)	189/928	19 (14-24)
Oio	3/494	0.6 (0.2-1.7)	11/655	1.5 (0.8-2.8)
Quinara	3/333	0.7 (0.2-2.2)	8/533	1.5 (0.7-3.2)
SAT	4/349	1.0 (0.4-2.7)	7/804	1.0 (0.5-2.1)
Tombali	2/301	1.0 (0.3-3.9)	18/451	6.0 (3.3-11)
Total	**73/4 300**	**1.6 (1.1-2.2)**	**339/6 117**	**5.0 (4.1-6.1)**

**Weighted by regional population*