Molecular surveillance of antimalarial partner drug resistance in sub-Saharan Africa: a spatial-temporal evidence mapping study

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Supplementary Material

Contents

A. Supplementary methods

A.1 Search protocol. The search protocol is outlined here, following the framework for scoping reviews set out by the Joanna Briggs Institute, and had not previously been registered[1]. The full PubMed search string is as follows:

> (((*pfmdr-1*[tw] OR *pfmdr*[tw] OR *pfmdr1*[tw] OR *mdr1*[tw] OR mdr[tw] OR *mdr-1*[tw] OR "plasmodium falciparum multidrug resistance" OR "plasmodium falciparum multi-drug resistance" OR "plasmodium falciparum multidrug resistant" OR "plasmodium falciparum multi-drug resistant" OR *pfcrt*[tw] OR "plasmodium falciparum chloroquine resistance transporter") OR ((drug[tw] OR drugs[tw] OR antimalarial[tw] OR anti-malarial[tw] OR antimalarials[tw] OR anti-malarials[tw] OR antimalaria[tw] OR anti-malaria[tw]) AND (resistance[tw] OR resistant[tw]) AND malaria[tw])) AND (africa[mh] OR africa*[tiab] OR angola*[tiab] OR benin*[tiab] OR botswana*[tiab] OR burkina faso[tiab] OR burundi*[tiab] OR cape verde*[tiab] OR cabo verde*[tiab] OR cameroon*[tiab] OR central african republic*[tiab] OR chad*[tiab] OR comoros*[tiab] OR congo*[tiab] OR cote d'ivoire*[tiab] OR ivory coast*[tiab] OR democratic republic of the congo[tiab] OR djibouti*[tiab] OR egypt*[tiab] OR equatorial guinea*[tiab] OR eritrea*[tiab] OR eswatini*[tiab] OR swaziland*[tiab] OR ethiopia*[tiab] OR ethiopia*[tiab] OR gabon*[tiab] OR gambia*[tiab] OR ghana*[tiab] OR guinea*[tiab] OR guinea-bissau*[tiab] OR kenya*[tiab] OR lesotho*[tiab] OR liberia*[tiab] OR madagascar*[tiab] OR malawi*[tiab] OR mali*[tiab] OR mauritania*[tiab] OR mauritius*[tiab] OR mozambique*[tiab] OR namibia*[tiab] OR niger*[tiab] OR nigeria*[tiab] OR rwanda*[tiab] OR sao tome and principe*[tiab] OR senegal*[tiab] OR seychelles*[tiab] OR sierra leone*[tiab] OR somalia*[tiab] OR south africa*[tiab] OR swaziland*[tiab] OR eswatini*[tiab] OR south sudan*[tiab] OR sudan*[tiab] OR tanzania*[tiab] OR togo*[tiab] OR uganda*[tiab] OR zambia*[tiab] OR zimbabwe*[tiab]).

An equivalent string was used to search in Embase:

> (pfmdr-1 or pfmdr or pfmdr1 or mdr1 or mdr or mdr-1 or pfcrt or crt or "plasmodium falciparum multidrug resistance" or "plasmodium falciparum multi-drug resistance" or "plasmodium falciparum multidrug resistant" or "plasmodium falciparum multi-drug resistant" or "plasmodium falciparum chloroquine resistance transporter" or ((drug* or antimalaria* or anti-malaria*) and resistan* and malaria)).mp. and ('africa'/ or africa*.mp. or (angola* or benin* or botswana* or burkina faso or burundi* or cape verde* or cabo verde* or cameroon* or central african republic* or chad* or comoros* or congo* or cote d'ivoire* or ivory coast* or democratic republic of the congo or djibouti* or egypt* or equatorial guinea* or eritrea* or eswatini* or swaziland* or ethiopia* or ethiopia* or gabon* or gambia* or ghana* or guinea* or guinea-bissau* or kenya* or lesotho* or liberia* or madagascar* or malawi* or mali* or mauritania* or mauritius* or mozambique* or namibia* or niger* or nigeria* or rwanda* or sao tome* or senegal* or seychelles* or sierra leone* or somalia* or south africa* or swaziland* or eswatini* or south sudan* or sudan* or tanzania* or togo* or uganda* or zambia* or zimbabwe*).tw.).

A.2 Data abstraction. With the literature identified through our systematic search, we abstracted information on sampling design, laboratory methodology and author affiliations. For sampling methodologies, we abstracted data on study design (including recruitment of patients directly from a clinic or hospital for direct specimen analysis; recruitment of patients from schools; treatment efficacy studies; malaria-positive travelers reporting to health facilities outside of the country of infection origin; sentinel surveillance; or other; if necessary, one study could include a maximum of two study design categories), average sample size for all markers, and symptomatic status and age range of study participants. For laboratory methodologies, we abstracted data on genotyping assay (each study could include as many lab assays as were used in that study for analysis of *pfcrt* or *pfmdr1* genes only), inclusion of polyclonal or mixed genotypes, reporting of the multiplicity of infection, and all molecular markers tested. We also included information on the geographic affiliations of the first and last author, as well as the title of the journal for the published report; authors with multiple geographic affiliations or co-first authors were counted once in each category.

For recording geographic coordinates, surveys were excluded from spatial analyses if samples were taken from a geographic area larger than one square hectometer and could not be separated by site, excluding small island nations such as Comoros. If a survey was carried out at multiple locations within 100 km from one another, and prevalence could not be matched to individual sites, we associated that survey with the site where a majority of samples were taken; if that information was not available, we randomly selected one site and recorded its geographic coordinates. We used 100 km as a threshold over which we felt surveys had limited spatial accuracy or applicability.

A.3 Time series analyses. In assessing changes in molecular marker prevalence over time, we also considered the following nonlinear functions: polynomial regressions, spline regressions and generalized additive models. We examined fit by subsetting the data for training and predictions and compared RMSE and R_2 and by regressing predicted and observed values.

B. Supplementary results and discussion

B.1 Study selection. We identified 254 studies that met our selection criteria, including 50 studies that, as of our final search on December 10, 2019, were not included in the WWARN PDMS database (Additional File 1A). Our search captured additional publications for nearly every year of the study period (2004–2017, excluding 2012) and data from 22 countries (Fig. S2). For spatial analyses, we excluded all traveler studies (n=13) and select non-traveler studies (n=11) from spatial analyses because results could not be disaggregated by site as per the above criteria (Additional File 1B).

B.2 Study characteristics. Sampling methodology is summarized in Table S1. The majority of specimens came from cross-sectional clinic recruitment studies for the direct purpose of sample analysis for drug resistance (46%), followed by specimen collection from treatment efficacy studies (33%). The majority of specimens (76%) were taken from symptomatic individuals. Age groups were relatively proportionately represented to malaria risk groups in the studies, with children <18 years old receiving the largest proportion of sampling (54%). Only a minority of published data (16%) came from studies that explicitly described their activities as related to sentinel surveillance measures, i.e. those studies that were at designated sites for national surveillance or sites that had a continuous, long-term resampling effort.

Genotyping methodology is summarized in Table S2. Many studies (44%) utilized the restriction fragment length polymorphism (RFLP) assay, considered less sensitive than newer techniques. However, the proportion of studies using RFLP for at least a subset of samples decreased annually by 4% (R_2=0·51, p=0·0064). On the other hand, the second most common assay was sequencing of a subset or all specimens (27%), and the proportion of studies using sequencing for at least a subset of samples increased annually by 4% (R_2=0·74, p<0·0001). About a third of studies (28%) used a combination of techniques for marker analyses. Most studies reported multi-clonal infections (65%) from polyclonal samples but few reported the multiplicity of infection (17%).

Publication and author affiliations are summarized in Table S3. A majority of first authors (64%) were affiliated with African academic institutions whereas the majority of last authors were affiliated with either European (42%) or American (20%) institutions, 8% of those being doubly affiliated with African institutions. Less than a third of last authors, or 31%, were affiliated exclusively with African institutions. All studies with publicly available information were from published journals indexed in PubMed, with no reports from independent research or governmental organizations. These publications were most commonly published in *the Malaria Journal* (34%), followed by *Antimicrobial Agents and Chemotherapy* (11%) and *the American Journal of Tropical Medicine and Hygiene* (11%).

B.3 Discussion of study characteristics. We found that the sampling and genotyping strategies in place in sub-Saharan Africa may not be representative of the true parasite population and have the potential for substantial selection bias.[2] Most studies utilize convenience sampling, specifically by obtaining samples directly from health facilities or clinical trials, which may not represent the underlying population genetics of the parasite population. Select studies have found there is no difference in drug resistance prevalence between symptomatic (or clinic) and asymptomatic (or community) populations at small spatial scales.[3–5] However, as we and other studies have noted, the landscape of drug resistance can vary across time and space.[6–8] Therefore, molecular marker surveys may be more representative of the underlying parasite population if they are spread out widely and include both clinic and community settings.

Additionally, the most predominant genotyping methodology over this time period was the less sensitive technique of RFLP, yet as alternative options become more accessible in low-resource settings and higher-throughput, the number of studies that conducted consequently sequencing increased significantly during the study period. Finally, only about one-fifth of studies report the multiplicity of infection, or infection with multiple genetically-differentiated clones of malaria. The MOI is an important tool to accurately calculate the frequency of markers in a parasite population, and without it, the prevalence of mixed infections is less valuable.[9,10] RFLP may not be sensitive enough to detect minority clones; together with the lack of information on transmission intensity, the prevalence of antimalarial resistance from these studies may not reflect that of the underlying parasite population genetics.[10,11]

B.4 Nonlinear trends in marker prevalence. Aggregated across sub-Saharan Africa, we found that cubic splines demonstrated superior fit compared to linear regression, and that second-order polynomial regression for *pfcrt* 76T and third-order polynomial regression for *pfmdr1* 86Y and *pfmdr1* 184F were better fitting than linear regression (Fig. S4). We chose to visualize only linear models in the main text and in Fig. S3 for ease of interpretability and consistency.

C. Supplementary Tables and Figures

Table S1. Characteristics of study design and sample collection of extracted literature.

	Number of studies (Proportion)
Sampling strategy	
Cross-sectional: clinic recruitment	118 (0.46)
Cross-sectional: household surveys	40 (0.16)
Cross-sectional: school recruitment	9 (0.04)
Treatment efficacy study	84 (0.33)
Traveler study	13 (0.05)
Sentinel site surveillance	38 (0.15)
Other	2 (0.01)
Clinical status	
Symptomatic	189 (0.76)
Asymptomatic	18 (0.07)
Both	41 (0.17)
Demographics	
Children (<5 years old)	54 (0.24)
Youth (<18 years old)	66 (0.30)
Adults	13 (0.06)
Pregnant women	11 (0.05)
All ages	78 (0.35)
Sample size (median, 95% CI)	93 (12, 588)

Table S2. Characteristics of molecular analyses of extracted literature.

	Number of studies (Proportion)
Molecular assay	
Restriction fragment length polymorphism	147 (0.44)
Sequencing	93 (0.27)
Real-Time or Quantitative PCR	56 (0.18)
Ligase detection reaction with fluorescent microspheres	11 (0.04)
Other	24 (0.07)
Reporting of mixed infections	
Included as separate category	137 (0.54)
Included as aggregated	28 (0.11)
Excluded	83 (0.33)
Markers	
pfcrt K76T	219 (0.86)
pfcrt 72-76 haplotype	85 (0.33)
pfmdr1 N86Y, Y184F, and/or D1246Y	190 (0.75)
pfmdr1 copy number variation	49 (0.19)
Reporting of Multiplicity of Infection	45 (0.17)

Table S3. Author and publication affiliations of extracted literature.

	Number of studies (Proportion)
First author affiliation	
African institution	121 (0.48)
European institution	49 (0.19)
American institution	31 (0.12)
Co-affiliation or other affiliation	42 (0.21)
Last author affiliation	
African institution	80 (0.31)
European institution	89 (0.35)
American institution	49 (0.19)
Co-affiliation or other affiliation	23 (0.15)
Publication affiliation	
Malaria Journal	86 (0.34)
Antimicrobial Agents & Chemotherapy	29 (0.11)
American Journal of Tropical Medicine and Hygiene	27 (0.11)
Other	112 (0.44)

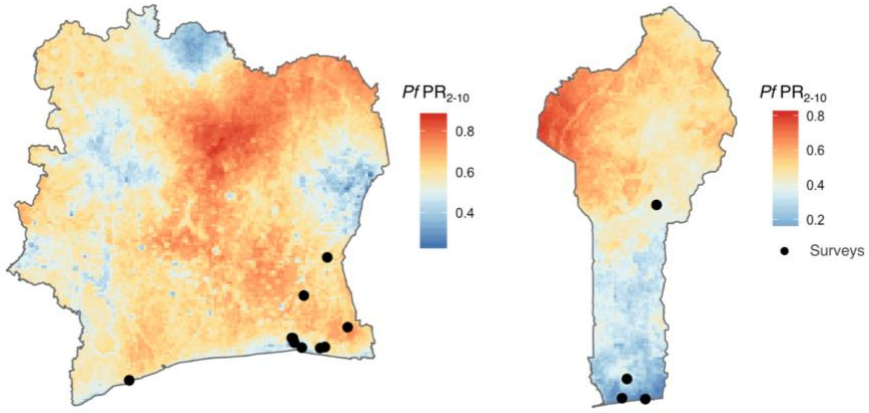

Figure S1. Examples of countries with geographically sparse molecular survey data on partner drug resistance: Côte d'Ivoire, left, and Benin, right. Dots are survey locations from 2004-2018, overlaid on an interpolated surface of malaria transmission intensity from the Malaria Atlas Project[12], measured as *Plasmodium falciparum* Parasite Rate[2-10] in 2010.

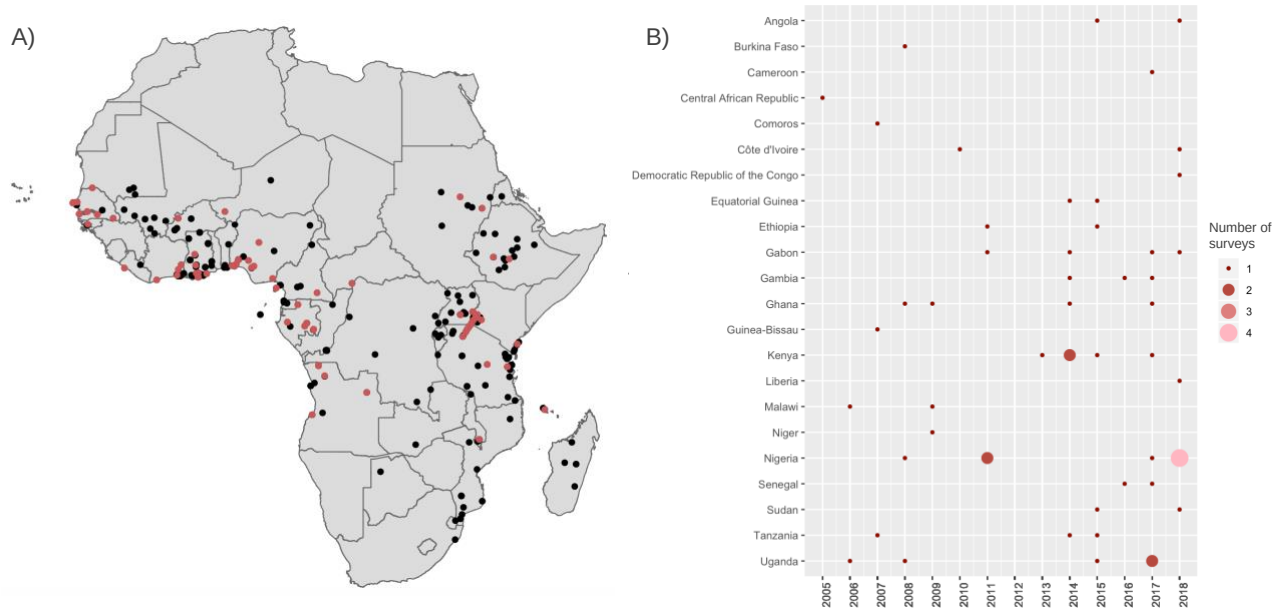

Figure S2. A) Locations of molecular partner drug resistance surveys in the PDMS (black points) and locations of additional surveys captured in our search (pink points) for the study period. B) Number of publications per country for each year of the study period. Publications shown here are only those additionally captured by our search (n=50), and not the publications that overlapped with those in the PDMS.

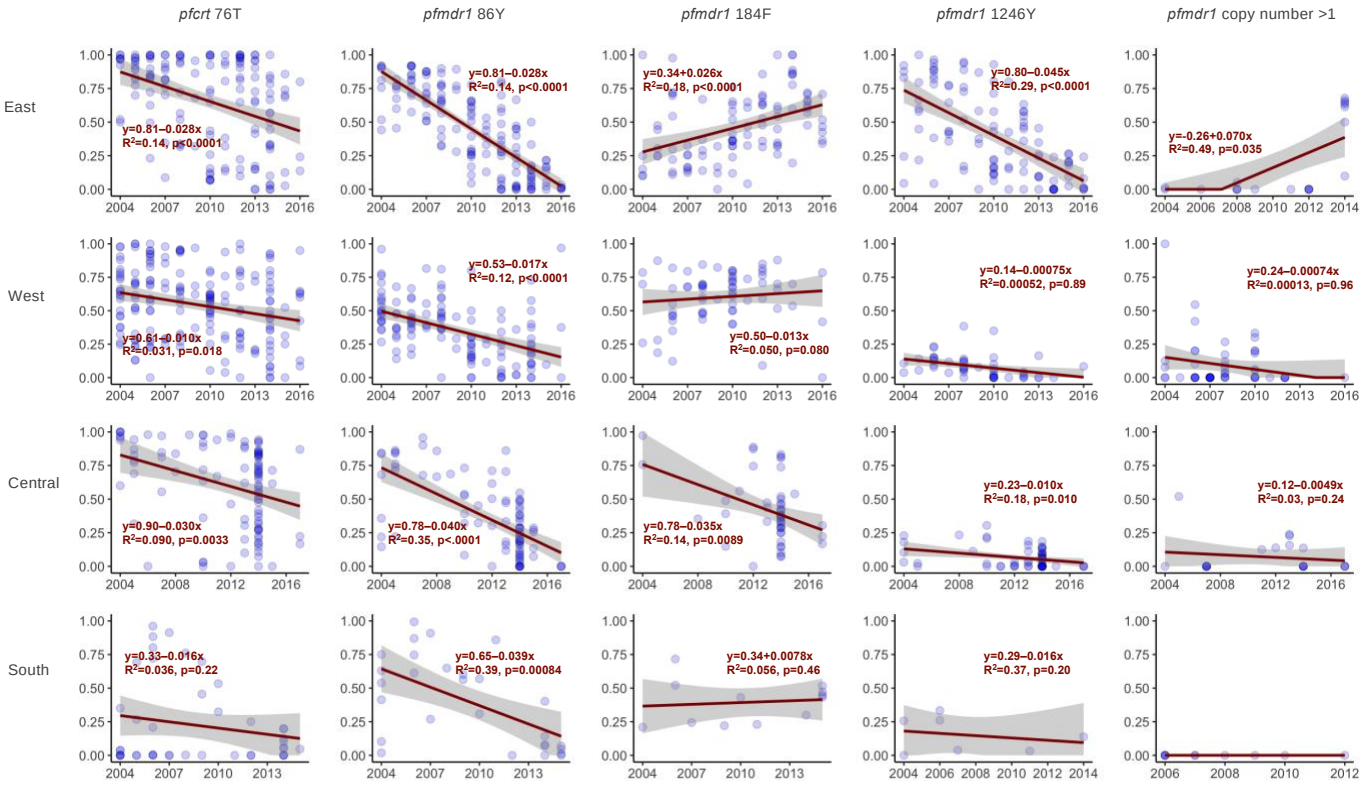

Figure S3. Time series of molecular marker prevalence (column) by UN subregion (row). Each blue point represents a single survey conducted the respective year and region; lines are best-fitting weighted linear regressions (dark red) with standard error bounds (gray).

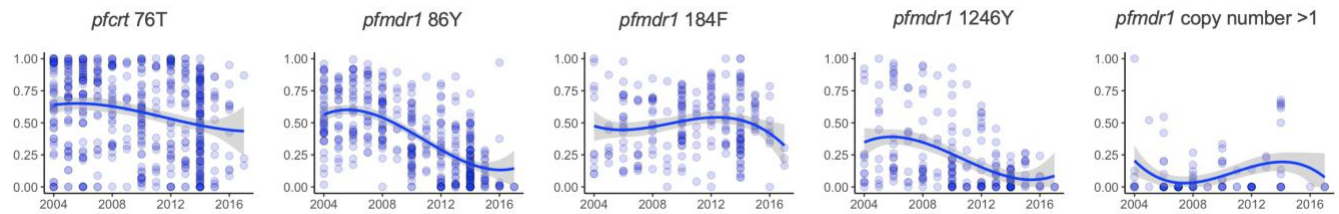

Figure S4. Time series of molecular marker prevalence (column) aggregated across sub-Saharan Africa. Each blue point represents a single survey conducted the respective year and region; lines are best-fitting cubic splines (blue) with standard error bounds (gray).

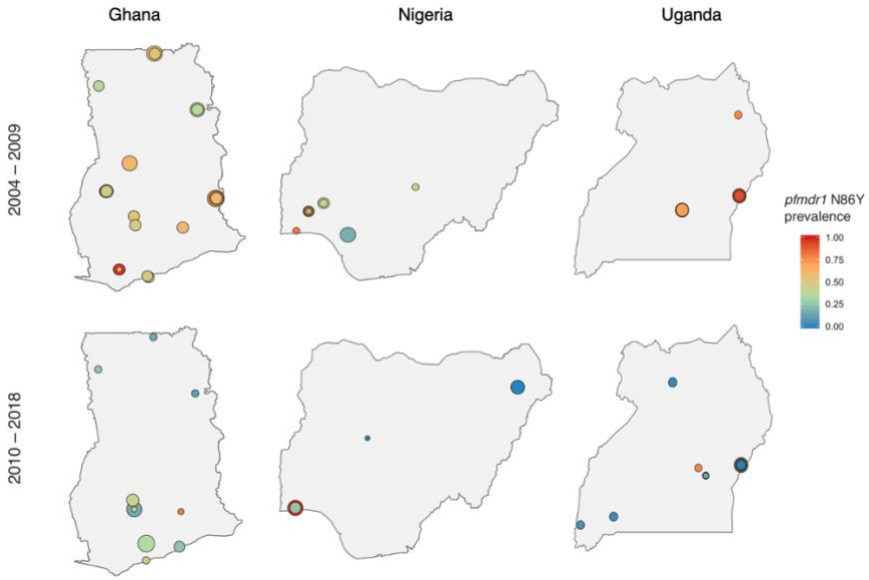

Figure S5. Prevalence of *pfmdr1* N86Y markers in Ghana (left), Nigeria (middle) and Uganda (right) for 2004-2009 (top row) and 2010-2018 (bottom row). The size of the point corresponds to the sample size and the color corresponds to the prevalence of mixed and/or mutant genotypes. The median and interquartile range of sample sizes for the three countries, respectively, are: n=47 (22), n=81 (31), n=124 (144). For the two respective time periods, Moran's I= 0·51 (p=0·0039) and -0·11 (p=0·98) in Ghana, 0·15 (p=0·29) and -0·21 (p=0·65) in Nigeria, and -0·13 (p=0·83) and -0·18 (p=0·17) in Uganda.

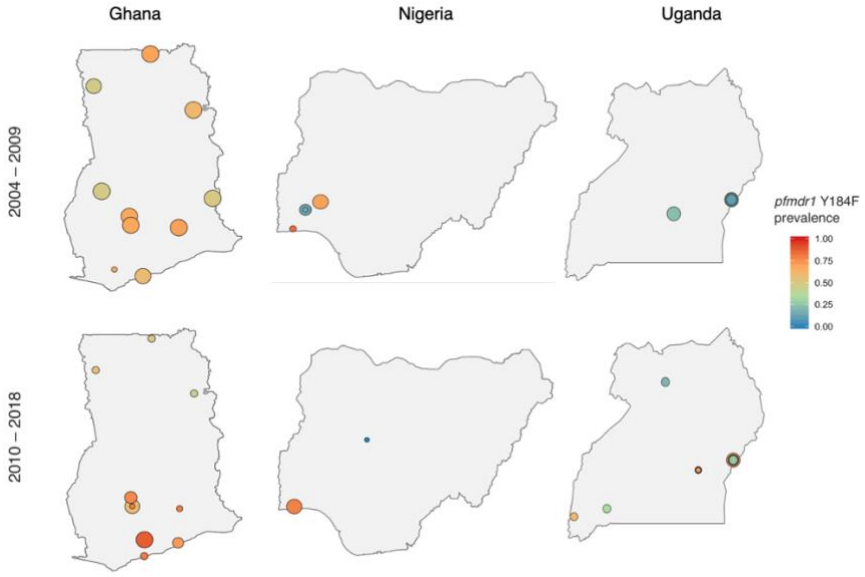

Figure S6. Prevalence of *pfmdr1* Y184F markers in Ghana (left), Nigeria (middle) and Uganda (right) for 2004-2009 (top row) and 2010-2018 (bottom row). The size of the point corresponds to the sample size and the color corresponds to the prevalence of mixed and/or mutant genotypes. The median and interquartile range of sample sizes for the three countries, respectively, are: n=40 (22), n=81 (31), and n=111 (134). For the two respective time periods, Moran's I= -0·06 (p=0·98) and 0·10 (p=0·28) in Ghana and -0·23 (p=0·60) and -0·05 (p=0·98) in Uganda, and for 2004–2009, Moran's I= 0·18 (p=0·16) in Nigeria.

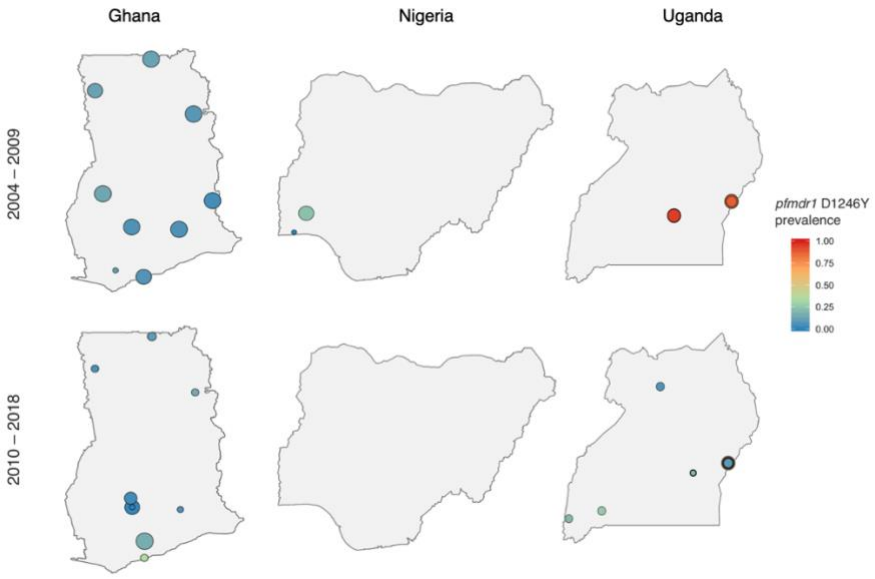

Figure S7. Prevalence of *pfmdr1* D1246Y markers in Ghana (left), Nigeria (middle) and Uganda (right) for 2004-2009 (top row) and 2010-2018 (bottom row). The size of the point corresponds to the sample size and the color corresponds to the prevalence of mixed and/or mutant genotypes. The median and interquartile range of sample sizes for the three countries, respectively, are: n= 40 (22), n=60 (31), and n=143 (140). For the two respective time periods, Moran's I= -0·22 (p=0·58) and 0·22 (p=0·04) in Ghana and -0·19 (p=0·62) and 0·04 (p=0·60) in Uganda. No data is available for Nigeria from 2010-2018.

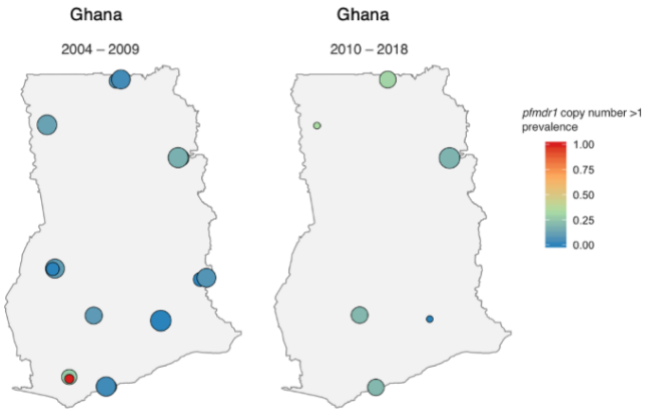

Figure S8. Prevalence of >1 copy of the *pfmdr1* gene in Ghana for 2004-2009 (left) and 2010-2018 (right). The size of the point corresponds to the sample size and the color corresponds to the prevalence of samples genotyped with >1 copy number. The median and interquartile range of sample sizes is n=12 (16). Moran's I= -0·20 (p=0·48) and -0·04 (p=0·15) for the two respective time periods.

D. References

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