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Supplementary Information for Mapping partner drug resistance to guide antimalarial combination therapy policies in sub-Saharan Africa

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Supplementary Methods

Covariate selection. We assessed variables related to transmission intensity, malaria intervention coverage, climate and population that we hypothesized may be associated with antimalarial drug resistance and for which we were able to obtain data at the relevant scale (more information in Table S1). To account for correlated variables in the analysis, we calculated pairwise correlations using the Spearman rank cross-correlation coefficients and collinear variables (r_s >0.50) were excluded based on model fit; if the coefficients of any pair exceeded 0.50, we used the covariate(s) producing the lowest deviance information criterion (DIC) in the Bayesian models. More specifically, seasonality was collinear with rainfall (r= -0.59) but contributed better fit. City accessibility was collinear with population density (r= -0.60), road quality (r= 0.56), and housing quality (r= -0.66), but produced the best model fit alone compared to inclusion of any combination of the other three variables. All uncorrelated variables were included in the final models.

Assessment of changes in prevalence over time. We determined the impact of covariates on the change in marker selection over time while accounting for estimation uncertainty from our logistic regression modeling. For a selected AD, we calculated the difference in prevalence estimates between the two time periods for all collected posterior samples, one posterior sample at a time. This process was repeated for all ADs and yielded 10,000 posterior samples of the change in prevalence over time in each AD. For a single set of differences across all ADs (i.e., one of the posterior samples), we fit a linear regression model with CAR random effects to determine which covariates explained variability in the estimated differences while also accounting for spatial correlation in the data. The model is given as:

$$Z_k^{(j)} \sim \mathrm{N}(\mu_k, \sigma^2) \text{ and } \mu_k = \mathrm{x}_k^{\mathrm{T}}\beta + \psi_k$$

where $Z_k^{(j)}$ is the difference in prevalence estimates from AD k and posterior sample j, and ψ_k and x_k have been previously described. We ran this analysis for a random subset of 100 of the posterior samples and estimated the regression parameters of interest and report the mean (across all 100 fitted models) posterior mean estimate as well as the mean upper and lower 95% credible intervals for all regression parameters.

Table S1. Population-level covariates hypothesized to be associated with antimalarial drug resistance. The table includes covariate names that are used in the main text, details and sources, as well as the possible directions and mechanisms of association with drug resistance, holding all other variables constant. WT=Wild-type; AL=artemether-lumefantrine; AS–AQ=artesunate-amodiaquine. AD= First-level administrative division.

Covariate name	**Description, units, source**	**Aggregation scale, year**	**Hypothesized association w/ frequency of mutant genotypes**	**Hypothesized mechanism**	**Included in spatial model**
Pf$PR_{2–10}$	*P. falciparum* parasite rate in children ages 2–10 years as a measure of transmission intensity (proportion) [1,2]	AD (2006, 2014)	Inverse	Increased parasite diversity leads to genetic recombination; population immunity may decrease drug pressure[12]	Yes
Pv$PR_{0–99}$	*P. vivax* parasite rate in individuals ages 0–99 years (proportion) [1,2]	AD (2006, 2014)	Positive	Availability of chloroquine to treat *P. vivax*	Yes
Rainfall	Annual precipitation (mm)[3,4]	AD (30-year average, 1970–2000)	Inverse	See *Pf*$PR_{2–10}$	No
Rainfall seasonality	Percentage of precipitation variability, also known as the coefficient of variation, calculated as the ratio of the standard deviation of the monthly total precipitation to the mean monthly total precipitation (%)*[3,4]	AD (30-year average, 1970–2000)	Inverse	Dry season w/o drug pressure leads to resurgence of WT alleles[13,14]	Yes
ACT coverage	Proportion of febrile, rapid-diagnostic-test (RDT) positive 0–5-year-old children treated with ACTs (proportion)[1,5]	National (2006, 2014)	Inverse (AL); Positive (AS-AQ)	AL selects for WT; AS-AQ selects for mutation[15]	Yes
ITN coverage	Insecticide-treated bed net (ITN) coverage (proportion)[1,6]	AD (2006, 2014)	Inverse (AL); Positive (AS-AQ)	Potentially linked to intervention allocation and ACT coverage	Yes
Population density	Population density adjusted by the United Nations World Population Prospects (log-transformed, persons per square km)[7]	AD (2005, 2015)	Positive	See *Pf*$PR_{2–10}$	No
City accessibility	Travel time to nearest city (log-transformed, min)[1,8]	AD (2015)	Unknown	Indicator of socioeconomic status and access to treatment[16]	Yes
Housing quality	Prevalence of improved housing: access to adequate water and sanitation, sufficient living area, durable construction (%)[1,9]	AD (2000, 2015)	Unknown	Indicator of socioeconomic status and access to treatment[16]	No
Road quality	Land-based travel speed (min)[1,8]	AD (2015)	Unknown	Indicator of socioeconomic status and access to treatment[16]	No
First-line drug policy	National first-line drug policy for confirmed P. falciparum malaria**[10,11]	National (2010)	Inverse (AL); Positive (AS-AQ)	AL selects for WT; AS-AQ selects for mutation[15]	Yes

* There are multiple proxies to represent seasonality. This particular metric may not exactly reflect areas receiving Seasonal Malaria Chemoprevention.
** For the policy in effect for the longest duration during the study period, with an average duration of 13 years. Once implemented, most countries (n=38/42) never changed their first-line ACT policy during the study period (see Fig. S2).

Table S2. Estimated prevalence for each marker and time period, aggregated by first-line therapy. The table shows the average fitted/estimated prevalence and standard deviation for all administrative divisions in the respective time period for countries using AL, AS-AQ, or both/other as first-line treatment, respectively.

Marker	Time period	Countries using AL (mean, SD)	Countries using AS-AQ (mean, SD)	Countries using both/other (mean, SD)
***pfcrt* 76T**	2004–2009	0.69 (0.21)	0.75 (0.20)	0.66 (0.15)
	2010–2018	0.44 (0.19)	0.53 (0.16)	0.41 (0.14)
***pfmdr1* 86Y**	2004–2009	0.62 (0.12)	0.67 (0.12)	0.57 (0.13)
	2010–2018	0.20 (0.13)	0.30 (0.14)	0.22 (0.13)
***pfmdr1* 184F**	2004–2009	0.48 (0.15)	0.51 (0.12)	0.59 (0.12)
	2010–2018	0.47 (0.15)	0.46 (0.15)	0.69 (0.10)
***pfmdr1* 1246Y**	2004–2009	0.26 (0.25)	0.30 (0.20)	0.10 (0.13)
	2010–2018	0.10 (0.09)	0.05 (0.04)	0.06 (0.07)

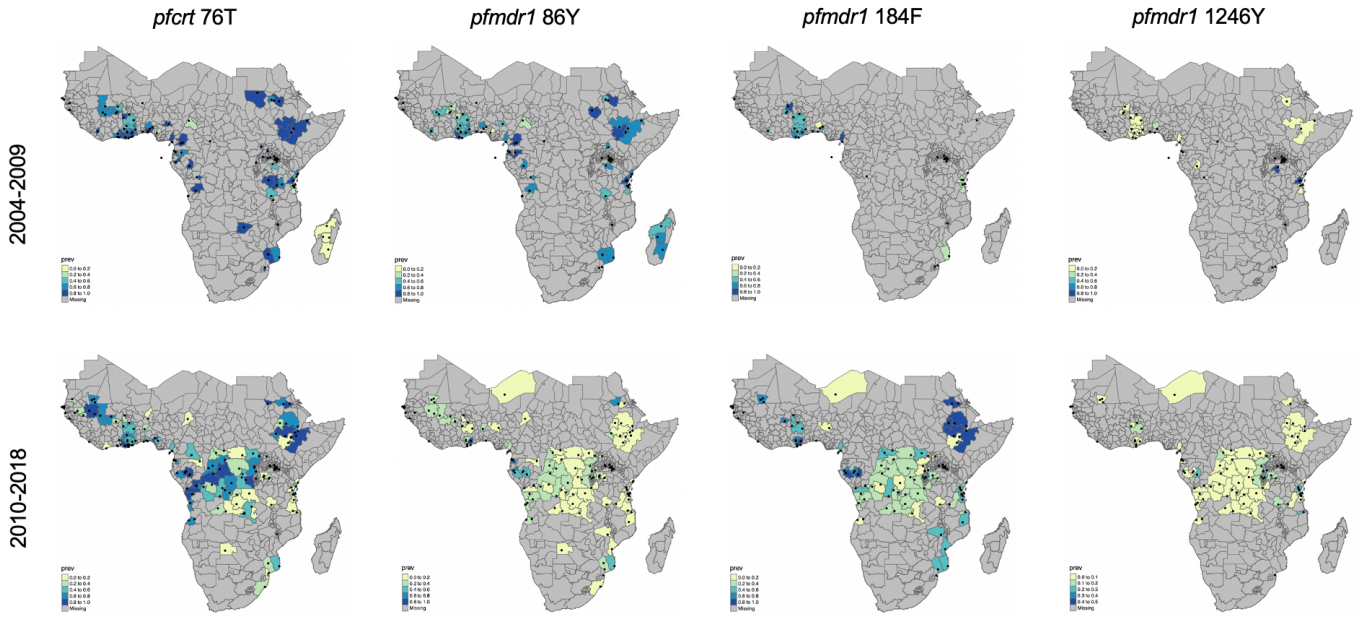

Figure S1. Observed prevalence of each mutation by first-level administrative division in 2004–2009 (top row) and 2010–2018 (bottom row), with black dots representing individual survey locations. Colors correspond to the degree of prevalence and were calculated as the weighted averages of all point surveys within a respective first-level administrative division and time period.

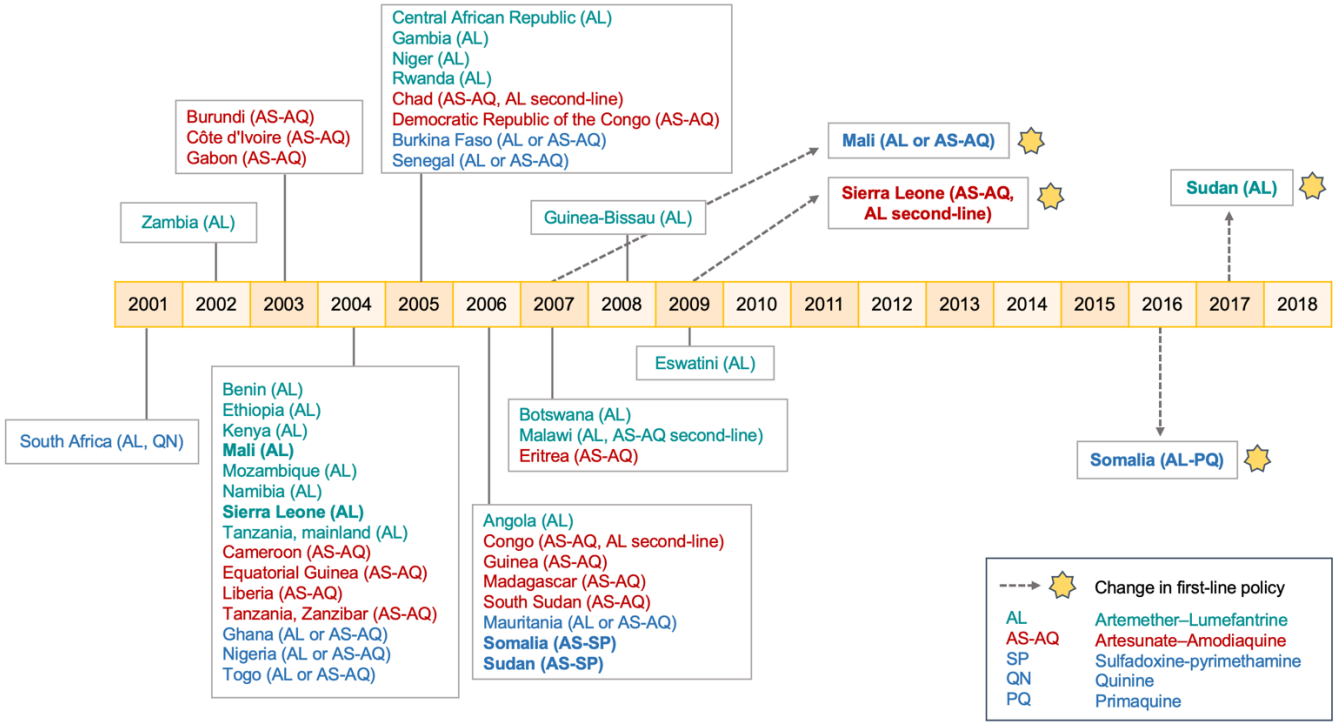

Figure S2. Timeline of the implementation of ACTs as national first-line antimalarial policies for the countries included in this analysis and study period. Dashed arrows with yellow stars represent changes to first-line ACT policy. Countries are color-coded by first-line ACT for ease of reference: AL (turquoise), AS-AQ (red), or both/other (blue). Information extracted from malaria country profiles (WHO): https://www.who.int/malaria/publications/country-profiles/en/. Our study categorized countries by first-line policy (AL, AS-AQ, or both/other) based on the first-line regimen used with the longest duration and widest coverage in the study period. Therefore, Sierra Leone was classified as AS-AQ, Tanzania as AL, and Sudan, Somalia and Mali as both/other.

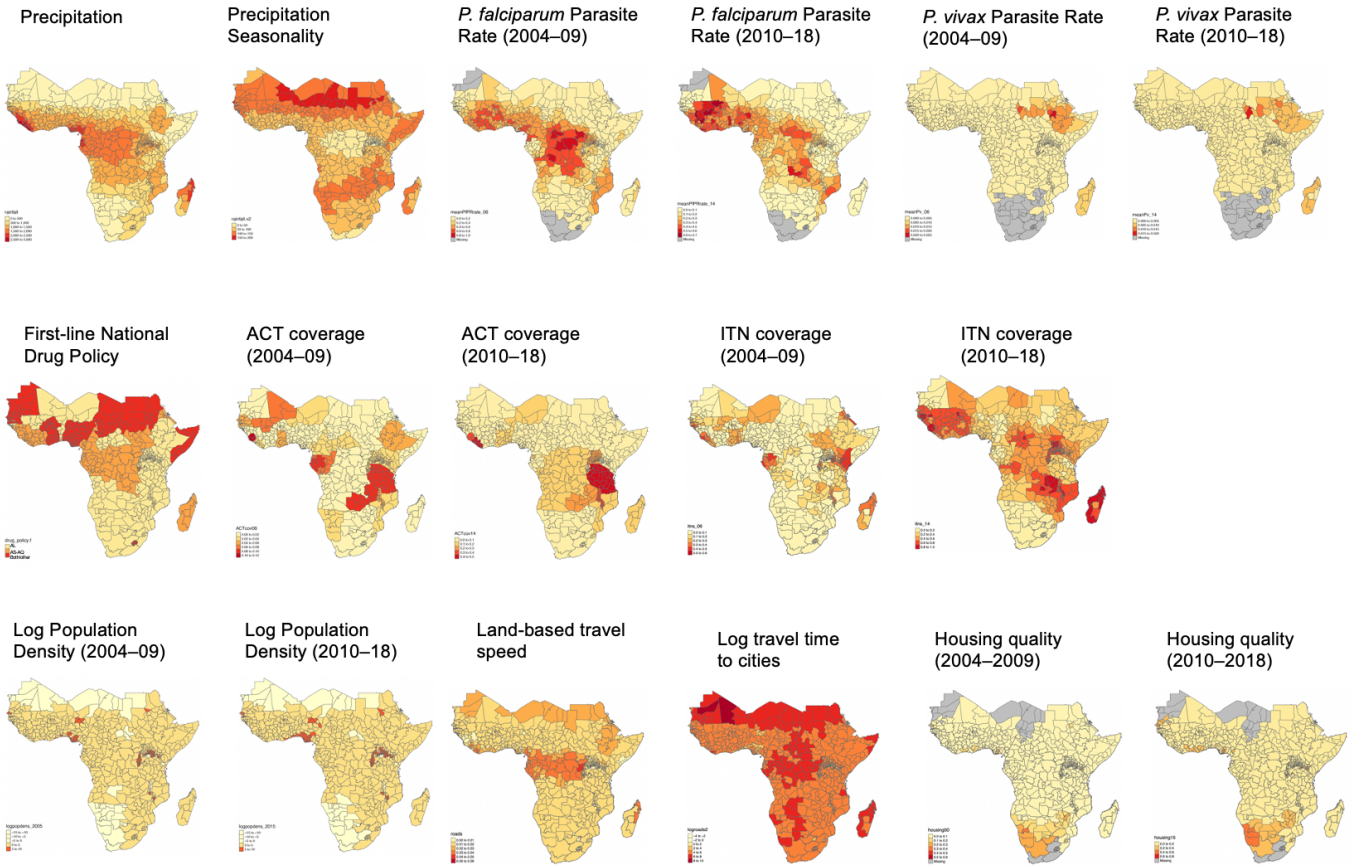

Figure S3. Covariates considered for model inclusion. Data (raster or polygon) were aggregated by first-level administrative division, when possible, or by country.

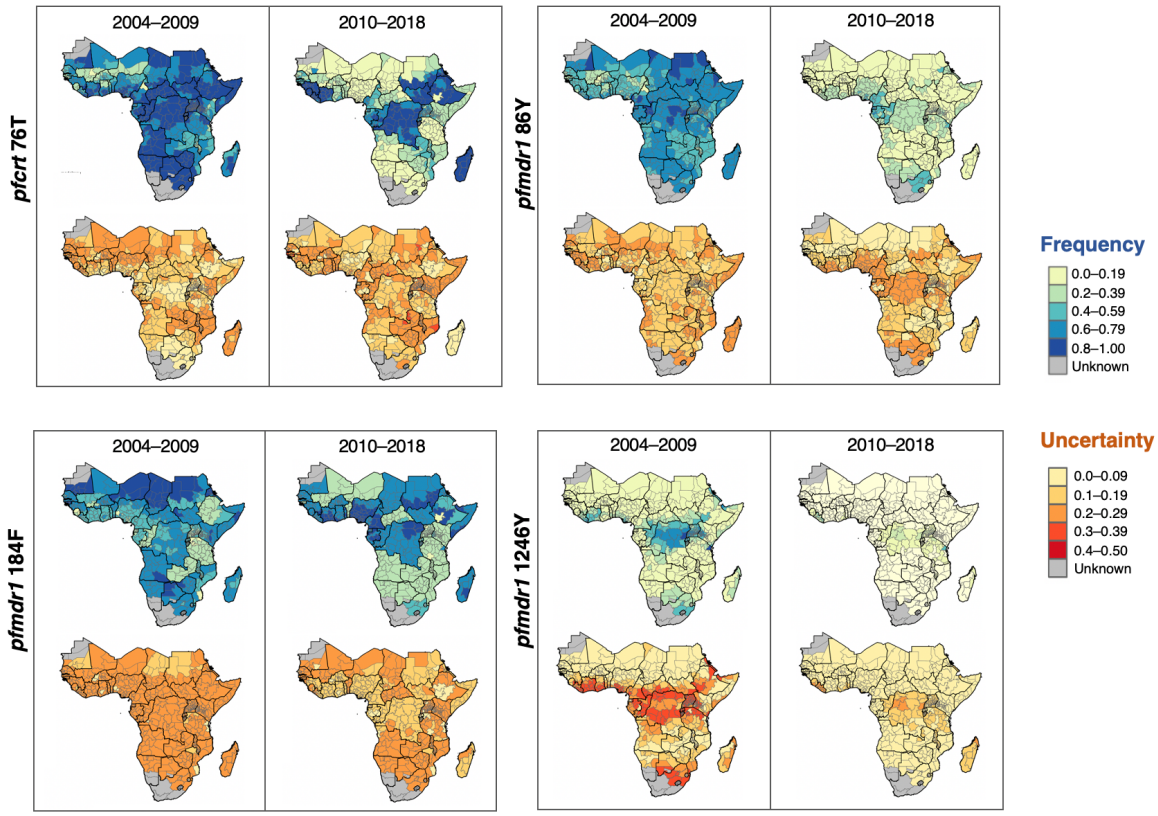

Figure S4. Posterior *frequency* estimates and standard deviations for 2004-2009 and 2010-2018 for each marker by first-level administrative divisions.

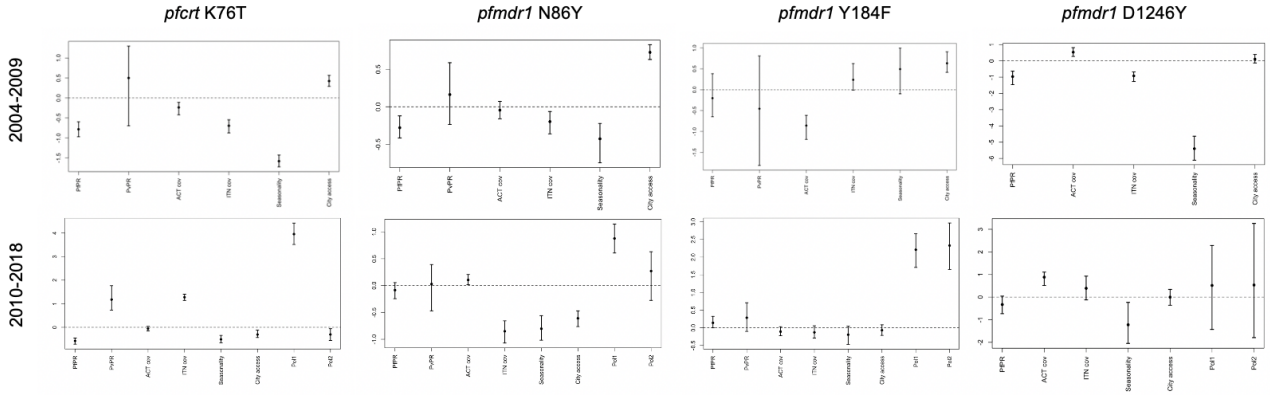

Figure S5. Posterior regression parameter estimates (median and 95% credible interval) for covariates used in spatial models estimating the *frequency* of individual molecular markers and time periods. Continuous variables are scaled for ease of comparison. Pol1= AS-AQ, Pol2= Both/other, Reference=AL.

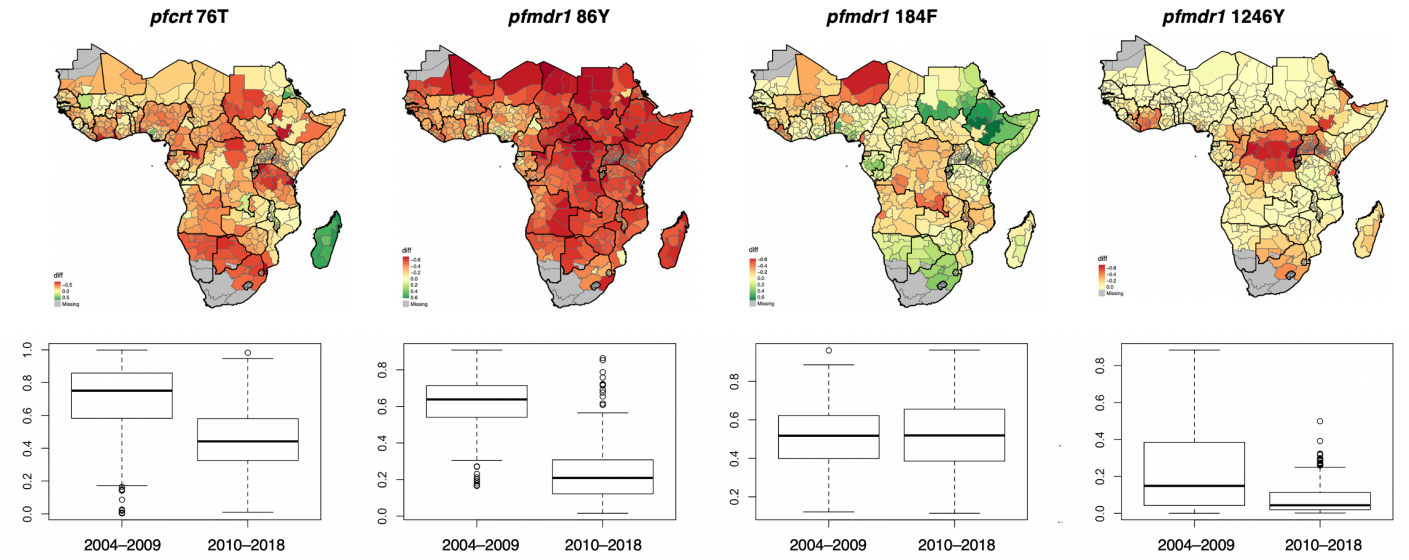

Figure S6. Difference in fitted/estimated prevalence from 2004–2009 to 2010–2018 by first-level administrative division. Red=decrease in prevalence, green= increase in prevalence (top row) with boxplots showing the distribution of fitted/estimated prevalence for all administrative divisions from 2004–2009 to 2010–2018 for each marker (bottom row).

Figure S7. Spearman rank cross-correlation coefficients for the change in prevalence for each pair of molecular markers using posterior prevalence distributions. Using univariate linear regression, every pair exhibited significant ($p<0.05$) associations. Notably, *pfmdr1* 86Y is significantly positively correlated with *pfmdr1* 184F, whereas *pfmdr1* 1246Y is positively correlated with *pfmdr1* Y184 ($p<0.0001$). In multivariate regressions, a decrease in pfcrt 76T was associated with a decrease in pfmdr1 86Y ($p<0.0001$), increase in 184F ($p<0.0001$), and non-significant decrease in 1246Y ($p=0.18$).

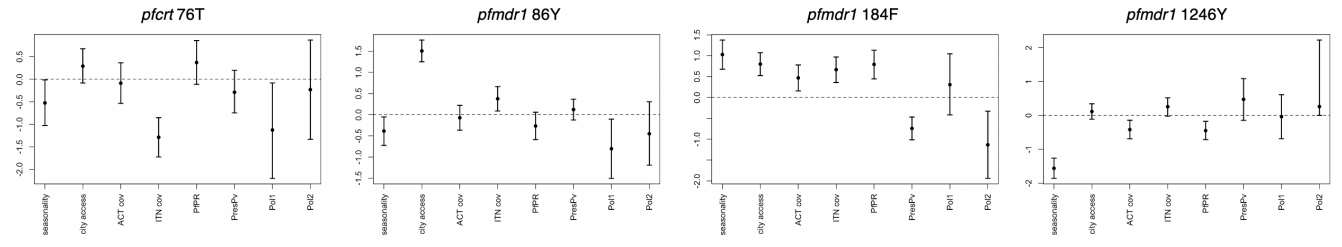

Figure S8. Posterior regression parameter estimates (median and 95% credible interval) for covariates used in spatial models estimating the change of individual molecular markers from 2004–2009 to 2010–2018. Continuous variables are scaled for ease of comparison. Pol1= AS-AQ, Pol2= Both/other, Reference=AL.

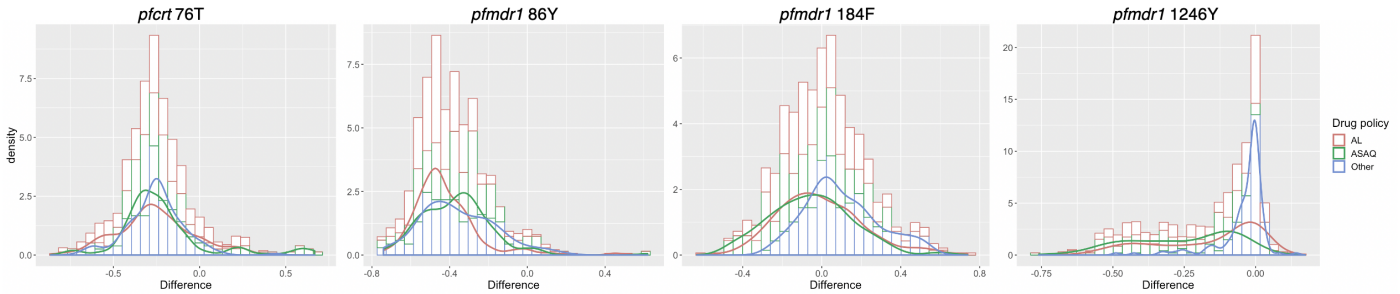

Figure S9. Histograms of the difference in fitted/estimate prevalence for first-level administrative divisions stratified by drug regimen. Colors correspond to first-line drug regimen, with red representing AL, green representing AS-AQ, and blue representing both/other, with lines displaying respective density curves.

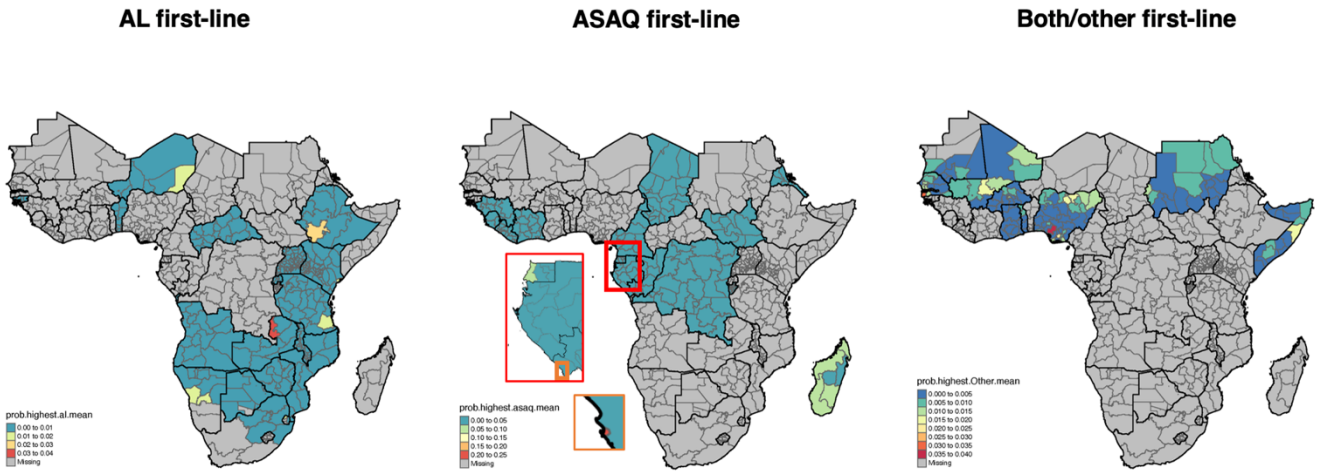

Figure S10. Posterior probability of selecting the region with the highest relative magnitude of change over 10,000 model iterations, averaged across all three markers. For AS-AQ, the city/region of Pointe-Noire in Congo is shown in the insets.

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