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SYSTEMATIC REVIEW

Rising trends of *Plasmodium falciparum* kelch13 mutations across East Africa (2014-2025): A decade review revealing critical surveillance gaps

[version 1]

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Abstract

The emergence and spread of *Plasmodium falciparum* resistance to antimalarial drugs has historically undermined malaria control efforts, with resistance to chloroquine and sulfadoxine-pyrimethamine first arising in Southeast Asia before spreading to Africa. Artemisinin-based combination therapies (ACTs), introduced in the mid-2000s, remain the cornerstone of malaria treatment; however, the emergence of artemisinin resistance (ART-R), associated with mutations in the *kelch13* (*k13*) gene, poses a growing threat. Increasing reports of *k13* mutations in sub-Saharan Africa, particularly in East Africa, raise concerns about the long-term efficacy of ACTs. This study synthesized available evidence on the spatial and temporal distribution of *k13* mutations in East Africa and assessed patterns and gaps in malaria molecular surveillance (MMS) and reporting. A systematic literature review was conducted across PubMed, Embase, Scopus, and Google Scholar to identify studies published between January 2014 and December 2025 reporting *k13* mutations in 13 East

African countries. A total of 2,532 records were identified, of which 126 studies met the inclusion criteria. Over 70,000 samples were analysed, with the highest contributions from Tanzania, Democratic Republic of Congo (DRC), Kenya, Rwanda, and Uganda, reflecting uneven surveillance intensity. Tanzania exhibited the highest diversity of WHO-validated *k13* mutations (six), followed by Kenya (five), while Rwanda and Uganda shared four mutations each. Ethiopia also reported four mutations, though with a distinct profile. Mutation prevalence and diversity increased after 2020, with peak prevalence reaching approximately 15% in Uganda (2022) and 10% in Rwanda (2024). Spatial clustering of mutations, such as R622I in the Horn of Africa and A675V, C469Y, and R561H in East Africa, suggests both local emergence and regional spread. In conclusion, ART-R in East Africa is an emerging and evolving threat. Strengthening standardized MMS, improving data reporting, and enhancing regional collaboration are critical to support early detection and preserve ACT efficacy.

Keywords
Plasmodium falciparum, kelch13 mutations, artemisinin resistance, malaria molecular surveillance, East Africa, antimalarial drug resistance, ACT efficacy, genomic epidemiology, mutation prevalence, spatial-temporal trends

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Introduction

The introduction of every new antimalarial drug has eventually been followed by the emergence of parasite resistance, which continues to pose a major challenge to malaria control and elimination efforts. Chloroquine resistance was first detected in Southeast Asia (SEA) in the 1950s and driven by mutations in the *Plasmodium falciparum* chloroquine resistance transporter (*Pfcrt*) gene. Resistance spread to Africa and other continents, leading to its withdrawal as first-line treatment in the early-1990's through the early 2000s.[1–5] Its replacement, sulfadoxine-pyrimethamine (SP), was adopted as an interim first-line treatment between the late 1990s into the mid 2000s[4,6,7] but was soon discontinued due to resistance driven by mutations in the dihydrofolate reductase (*dhfr*) and the dihydropteroate synthase (*dhps*) genes.[8,9] In the mid-2000s, most African countries introduced artemisinin-based combination therapies (ACTs) as the first line treatment. Deployment of efficacious ACTs was a contributor to the significant reductions in malaria burden between 2000 and 2015. However, artemisinin partial resistance (ART-R) emerged in South East Asia in 2006 (ART-R)[10,11] and was followed by resistance to partner drugs leading to ACT treatment failure.[12,13] Clinically, ART-R manifests as delayed clearance of ring-stage parasites *in vivo* and is linked to mutations in the parasite's kelch13 (*k13*) propeller domain.[14]

Over the past decade, several WHO-validated *P. falciparum k13* mutations have been detected in sub-Saharan Africa (SSA), raising serious concerns about the long-term efficacy of ACTs. In East Africa, emerging evidence of validated *k13* mutations from Rwanda,[15,16] Ethiopia,[17,18] Uganda,[19–21] Tanzania,[22,23] and Kenya.[24,25] Based on the analyses of the sequence surrounding the *k13* mutations, the findings indicate independent emergence of ART-R mutations in the region (i.e., not imported from South East Asia) and subsequent regional spread. Despite recent large-scale global syntheses of ART-R, including the analyses of over 100,000 *P. falciparum* samples by Balmer et al., (2025),[26] important regional gaps remain. Such studies primarily provide broad spatiotemporal insights at global scale and may not fully capture fine-scale heterogeneity within specific regions. In particular, relatively few studies have synthesized the status of ART-R at regional level in Africa, limiting the ability of National Malaria Control Programs (NMCPs) to make fully informed policy decisions. Amid the emergence of ART-R and the challenges posed by coverage of malaria molecular surveillance (MMS) and inconsistent reporting, there is a pressing need to consolidate existing evidence to guide timely action by NMCPs and regional stakeholders. To address this gap, this review collates *k13* genomic data across both space and time, providing insights into temporal and geographic trends of *P. falciparum* resistance in East Africa. Drawing on studies conducted in malaria-endemic countries from 2014 to 2025, this review aims to generate a consolidated report on *k13* mutations to offer evidence-based guidance to NMCPs.

The World Health Organization (WHO) recommends standardized reporting of *P. falciparum* resistance markers and encourages national programs to integrate molecular methods into routine surveillance, as outlined in the WHO compendium of molecular markers for antimalarial drug resistance (AMDR).[27] Common inconsistencies such as variations in genotyping methods, differences in study design, missing data, and under-reporting, can complicate comparisons of prevalence estimates and limit accurate assessment of molecular markers AMDR. This underscores the need for standardized and early reporting of molecular markers of AMDR to enable reliable comparisons across regions and over time.

This review therefore provides an updated overview of the status of ART-R in Eastern Africa and informs the development of a harmonized framework for monitoring and reporting of AMDR, to strengthen surveillance and particularly MMS efforts and support evidence-based policy decisions in malaria-endemic countries.

Methodology

Search strategy

A literature search was conducted on four databases: Google Scholar, Embase, PubMed, and Scopus, to identify peer-reviewed studies reporting *k13* mutations in East African countries over the previous 12 years, between January 1st, 2014, and December 31st, 2025. This period was selected to capture the emergence and subsequent evolution of *k13* polymorphisms in the region, as earlier studies reported minimal or no variation prior to 2014.[15] The following search terms were used: "((malaria OR *falciparum*) AND (molecular markers OR *kelch13* OR *Pfkelch13* OR *k13* OR *PfK13*) AND (Kenya OR Uganda OR Ethiopia OR Somalia OR Rwanda OR Burundi OR Tanzania OR Zanzibar OR Congo OR DRC OR Eritrea OR Djibouti OR Sudan OR South Sudan)) AND (("2014/01/01" [Date - Publication]: "2025/12/31 " [Date - Publication]))".

Eligibility criteria

This review covered studies published in English between 1st January 2014 and 31st December 2025 that reported the prevalence of *k13* mutations in Eastern Africa countries. Eligible studies were those on human participants of all ages and must have explored symptomatic, asymptomatic or uncomplicated malaria cases. For a study to be included in the review, it must have reported the frequency or prevalence of *k13* mutations using molecular genotyping methods. Studies were

excluded from the review based on the following criteria: abstracts with no full texts, conducted on populations outside East African countries, reviews, case reports, meta-analysis, *in vitro* studies, studies with no *k13* genotyping data, non-*P. falciparum* studies, and no full text.

Review process

A total of 2,532 records were identified from searches conducted in the four databases. After removal of 300 duplicates, 2,232 unique records remained for screening. Four reviewers (EA, JM, JK and RK) independently screened these records to assess them for eligibility based on titles and abstracts. Consequently, 2,012 irrelevant records were excluded, and full text articles were sought for the remaining 220 records. However, five articles could not be retrieved because their full text versions could not be reliably traced, therefore, 215 articles were available for full text screening. After careful review, 89 articles were excluded for the following reasons: 15 were conducted outside Eastern African countries, 37 lacked *k13* genotyping data, 33 were of wrong publication types (including theses, preprints, dissertation, case reports, reviews, and abstracts), 3 used data from existing databases, and 1 was an *in vitro* study (Figure 1). A total of 126 articles proceeded to data extraction and downstream analysis (see Supplementary Figure 1 for a summary of studies per country).

Data extraction procedure

The following details were extracted from the full texts: year of sample collection, year of publication, country of origin, region/province/town, age of study participants, sample size, first- line ACT recommended in the country of origin, genotyping assay, study population (asymptomatic or symptomatic), sample successfully sequenced, *k13* validated, and candidate mutations and their frequencies. A fifth reviewer (LIO) assessed the extracted data for correctness, completeness, and consistency. Some studies included samples from more than one country; in such cases, samples were counted separately for each country where disaggregated data were available.

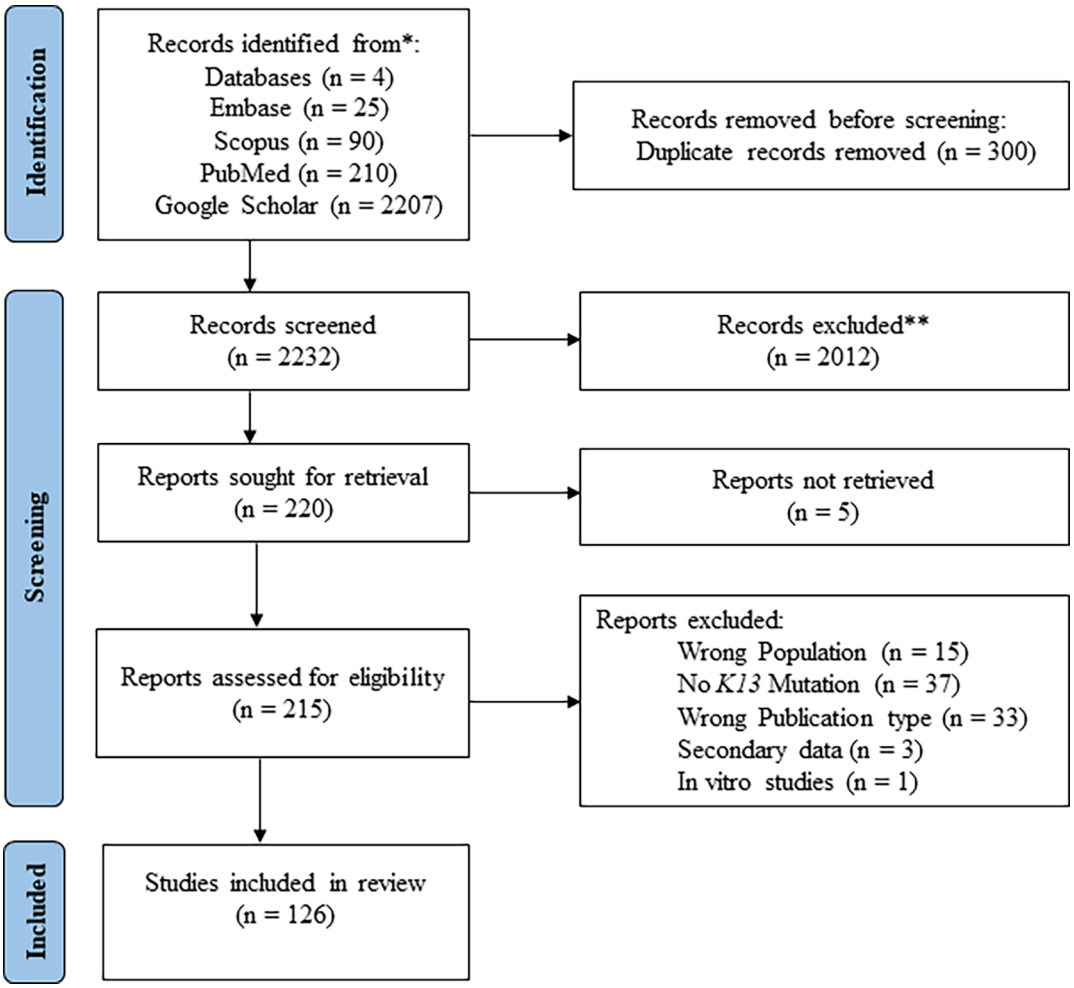


Figure 1. PRISMA flow diagram illustrating the study selection criteria.

Data analysis

Pre-analysis data processing

Prior to statistical analysis, extensive data cleaning and preparation were carried out using Python within a Jupyter Notebook environment. Individual sheets compiled by the four reviewers each containing information on either validated, candidate or other non-synonymous and synonymous *k13* mutations were merged into a unified dataset. The combined data were further enriched by incorporating geographic coordinates based on the "country" and "town" columns that showed the location of each study. Standardization procedures were applied to ensure consistency across string-based columns, including stripping unnecessary whitespace and formatting mutation names uniformly so that each mutation type was represented on its own row. To support frequency-based analysis, two key metrics were computed: the raw count and percentage frequencies of the different mutation. This was derived from the frequency and samples-genotyped columns and to ensure data uniformity and analytical accuracy across the subsequent visualization and statistical procedures.

Data analysis and visualization

All data analysis and visualization were conducted using python3. Numerical operations and manipulation were performed using *NumPy* and *Pandas* libraries. For data visualization and statistical plots, we employed *Matplotlib* and *Seaborn* libraries and their related packages. Geospatial analysis and the generation of maps were carried out using G*eoPandas* with supplementary elements such as scale bars and annotation incorporated via M*atplotlib.patches.* The geo-coordinates used for the different locations were extracted from Mapcarta and LATITUDE and the shapefile from Natural Earth. Additional data wrangling, coordinates projection and spatial joins were also implemented within the G*eoPandas* framework. All analyses were conducted in a reproducible computing environment using jupyter notebook. The analysis scripts are publicly available in the Github repository k13_review.

Extracted data were collated and quantitatively synthesized to evaluate the temporal and spatial distribution of *k13* mutations across East Africa between 2014 and 2025. Descriptive analyses were performed using aggregated study-level data, including mutation frequencies, prevalence estimates and geographic distribution patterns. Figures and graphical summaries were generated from pooled extracted datasets to visualise spatio-temporal trends, surveillance coverage and the distribution of validated and other non-validate *k13* mutations across the region.

Results

Spatial distribution of *k13* mutations

To evaluate the spatial distribution of *k13* surveillance efforts, we summarised the total number of *P. falciparum* samples analysed per country between 2014 and 2025 (Supplementary Table 1, Figure 2). The largest number of genotyped samples were reported from Tanzania, Kenya, Uganda and the Democratic Republic of Congo (DRC). In contrast, countries such as Burundi, Eritrea, Somalia, South Sudan, Sudan, and Zanzibar contributed relatively fewer samples. Notably, no *Pfk13* data were identified from Djibouti during the review period.

Overall, there was a wide variation in surveillance intensity across countries and years, with sustained sampling in Tanzania, Kenya, DRC, Rwanda, Uganda and Ethiopia, and sparse sampling in the other countries.

The distribution and diversity of *k13* mutations including validated (Supplementary Table 2), candidate (Supplementary Table 3), potential (Supplementary Table 4) as per WHO definition, and other non-synonymous and synonymous mutations (Supplementary Table 5) varied across ten East African countries. Tanzania exhibited the widest range of WHO-validated mutations, with 6 mutations (A675V, C469Y, M476I, P574L, R561H, and R622I), followed by Kenya with five (A675V, C469Y, P553L, R539T and R561H). Interestingly, Rwanda and Uganda reported an identical set of four validated mutations (A675V, C469Y, P574L, and R561H). Ethiopia also reported four validated mutations, although with a different type of mutation profile (P574L, C580Y, R622I and A675V). Fewer validated mutations were observed in other countries including Sudan and DRC (three mutations each), Eritrea (two mutations), Zanzibar (R561H) and Somalia (R622I) (Supplementary Table 1, Figure 3). The regional distribution (Figure 5B) revealed distinct geographic clustering of specific alleles. R622I predominated in the Horn of Africa, Ethiopia, Eritrea, Somalia and Sudan, while A675V and C469Y dominated in Kenya and Uganda and R561H, was predominant in Tanzania and Rwanda. The DRC, on the other hand, was largely characterized by A675V. South Sudan and Burundi reported no validated mutations, and Djibouti lacked any published data.

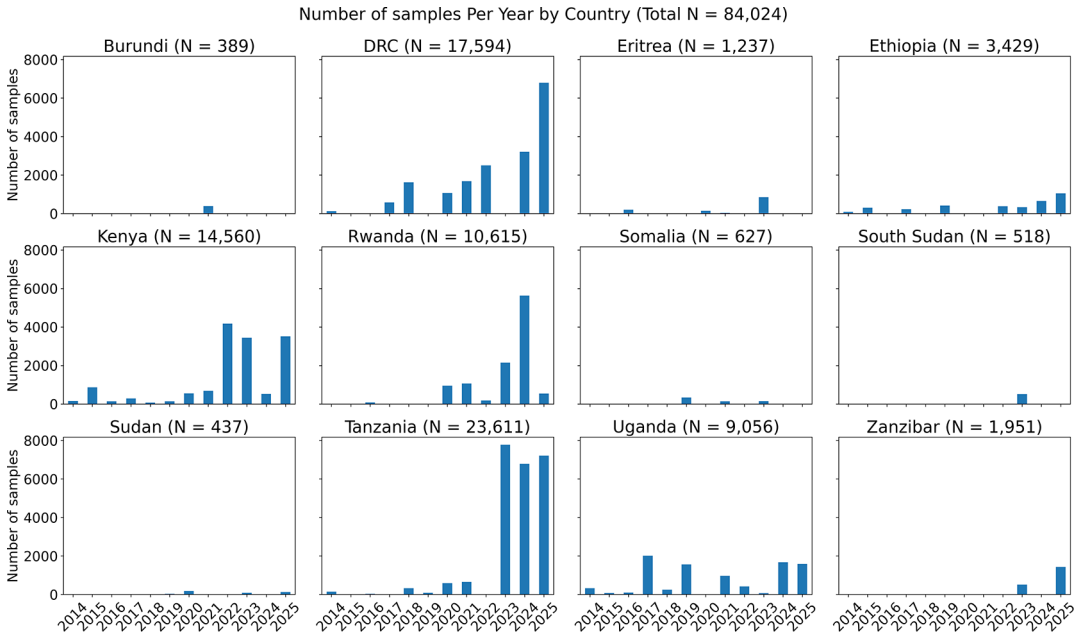


Figure 2. Total number of *P. falciparum* samples genotyped for *k13* mutations between 2014 and 2025 across 12 East African countries, shown with their temporal distribution. The highest number of samples were analysed in Tanzania (n = 23,611), Kenya (n = 14,560), Rwanda (n = 10,615), Democratic Republic of Congo (n = 17,594) and Uganda (n = 9,056), collected across multiple years with notable peaks from 2023 to 2025. In contrast, Burundi, Eritrea, Ethiopia, Somalia, South Sudan, Sudan and Zanzibar contributed comparatively fewer samples with the sampling largely concentrated in one or two years. No studies from Djibouti were identified.

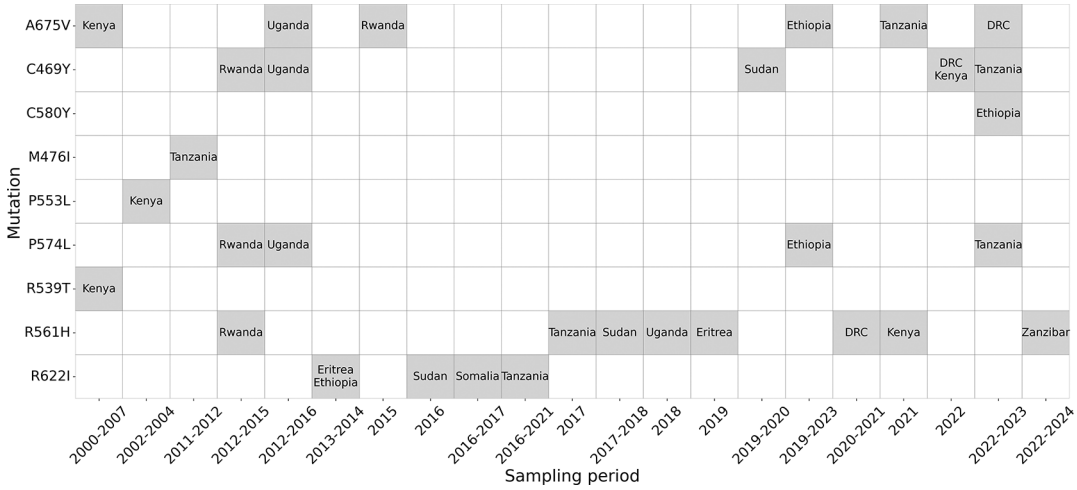


Figure 3. Spatiotemporal emergence and regional expansion of WHO-validated *k13* mutations in East Africa. The figure summarises the earliest documented detections of specific *P. falciparum k13* mutations across the study region. The horizontal axis represents sampling years or ranges as reported in the original source literature. Where studies reported mutations over aggregated sampling intervals (e.g., 2000–2007) without specifying the exact year of first detection, the full sampling range is retained as a discrete temporal category. The vertical axis lists the WHO-validated *k13* mutations identified in the analysed data set. Each grey cell indicates the earliest documented detection of a given mutation in a specific country during that sampling period. Where the same mutation was first reported in multiple countries within the same period, all relevant countries are listed within a single cell. Only sampling periods corresponding to first detections for each country-mutation combination are shown, emphasizing temporal emergence and subsequent geographic spread. White (blank) cells indicate no first detection reported for that mutation during that sampling period within the analysed datasets.

Publication Year	DRC	Eritrea	Ethiopia	Kenya	Rwanda	Somalia	Sudan	Tanzania	Uganda	Zanzibar	Total
2014	0	-	0	1(0.5)	-	-	-	0	0	-	**
2015	-	-	1(8.9)	0	-	-	-	-	0	-	**3**
2016	-	0	-	0	2(1.5)	-	-	0	0	-	**2**
2018	0	-	-	0	-	-	-	0	2(0.5)	-	**2**
2019	-	-	0	0	-	1(0.7)	0	0	3(1.8)	-	**13**
2020	0	1(0.8)	-	0	3(0.8)	-	0	1(0.9)	-	-	**27**
2021	0	0	0	0	4(4.6)	1(0.7)	-	1(0.4)	2(6.6)	-	**156**
2022	0	-	0	0	2(11.6)	-	-	-	2(13.2)	-	**44**
2023	-	2(4.4)	1(9.6)	3(4.1)	3(8.4)	0	3(3.0)	3(0.6)	3(4.7)	0	**876**
2024	3(1.3)	-	1(20.7)	2(15.6)	3(14.6)	-	-	5(6.7)	3(6.8)	-	**2289**
2025	0	1(18.1)	4(17.6)	4(3.8)	3(9.7)	-	1(19.8)	1(9.2)	3(13.1)	1(0.9)	**1068**

Figure 4. Spatiotemporal distribution of validated *k13* mutations across East Africa. The heatmap summarises the spatiotemporal trends of validated k13 single nucleotide polymorphisms (SNPs) reported across ten East African countries between 2014 and 2025. Each coloured cell displays two values, the total number of unique validated *k13* SNPs identified in that country and year (left) and in parentheses (), the proportion (%) of sequenced samples carrying validated *k13* mutations (right). The colour intensity corresponds to the number of unique validated mutations detected, with darker blue indicating greater diversity of validated mutations. Cells marked with a dash ("–") indicate years for which no genotyping data were available, while "0" indicates years in which samples were analysed but no validated *k13* mutations were detected. The Total column represents the total number of validated *k13* mutations reported across all included countries for each publication year. ** For 2014, the reported proportion was calculated based on sequencing reads rather than the number of samples, and therefore may not be directly comparable with proportions reported for other years.

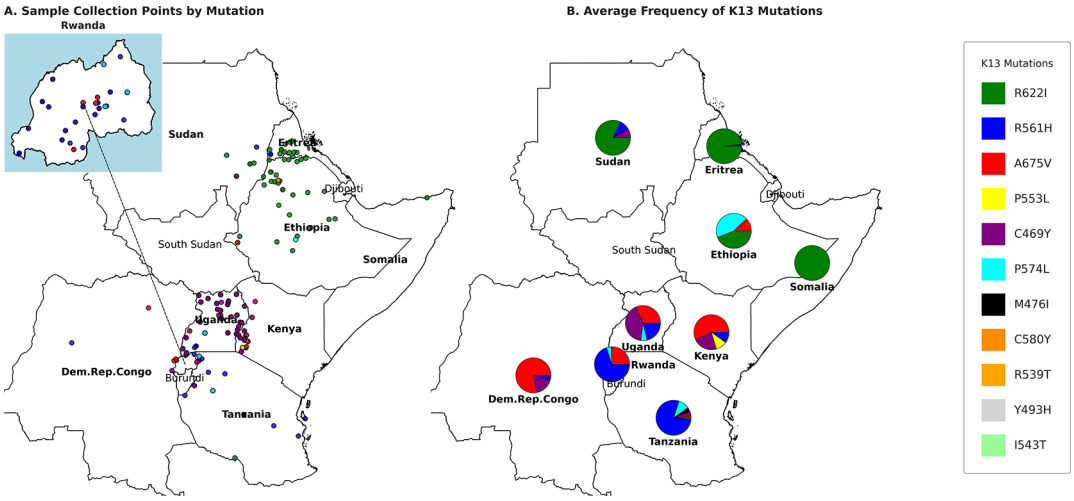


Figure 5. Regional distribution and sampling sites of validated *k13* mutations across East Africa. (A) Spatial distribution of *P. falciparum k13* sampling sites across East Africa. Each point represents a collection location coloured by the detected WHO-validated mutation. An inset highlights dense sampling in Rwanda. Sampling was not uniformly distributed across the countries. (B) Average frequencies of WHO-validated *P. falciparum k13* ART-R conferring mutations across ten East African countries. Pie charts are overlaid on each country, with coloured segments representing the mean frequency of a specific validated mutation. Uganda showed higher frequencies of C469Y (purple), while Rwanda and Tanzania were dominated by R561H and Kenya majorly dominated by A675V. DRC primarily harbored A675V, while R622I predominated in Ethiopia, Eritrea, Somalia and Sudan. Blank regions indicate the absence of validated mutations or unavailable data. South Sudan and Burundi reported no validated mutations, and no data were available for Djibouti.

Temporal distribution

The earliest reported sampling year for the identification of WHO-validated *k13* artemisinin conferring mutations across the ten East Africa countries was between 2000 and 2007 in Kenya (Figure 3). In Kenya, validated mutations such as R539T, A675V, and P553L were identified as early as 2000–2007 and 2002–2004, respectively. Subsequently, Tanzania reported the emergence of M476I mutation during 2011–2012. In Rwanda, multiple validated mutations including C469Y, P574L, and R561H were detected between 2012 and 2013, while Ethiopia reported R622I by 2013. Around the same period, Uganda reported the presence of A675V, C469Y, and P574L mutations across samples collected from 2012–2016.

Additionally, there was an increase in the diversity and prevalence of validated *k13* SNPs across the sampled East African countries over the last decade (Supplementary Table 2; Figure 4). In earlier years (up until 2020), the number of validated *k13* mutations was low with the proportion of affected samples rarely exceeding 1%. However, there is a shift from 2020, with an increased detection of the number of SNPs and their prevalence in several countries. By 2023 and 2024, several countries show a marked increase: Rwanda and Uganda consistently showed a high number, at least 1 of the validated mutations over time, with peak mutation prevalence reaching approximately 14.6% in Rwanda (2024) and 13% in Uganda (2022 and 2025). Ethiopia experienced a significant jump from 9.6% in 2023 to 20.7% in 2024. Tanzania in contrast had the highest number of unique validated mutations with five recorded in 2024, although the reported sample prevalence for that year was approximately 6.7% increasing to a peak prevalence of 9.2% in 2025. There was an overall trend of increasing sample prevalence of validated *k13* mutations in the East Africa region.

Sampling distribution

To visualise both the regional prevalence and spatial distribution of validated *P. falciparum k13* mutations, average mutation frequencies and sampling locations were mapped across East Africa (Figure 5A and 5B). Of note, sampling site distribution (Figure 5A) was uneven across the region. In DRC, most sampling occurred near Kinshasa and along the Rwandan border, whereas Rwanda demonstrated relatively uniform coverage nationwide. In Ethiopia, sampling was concentrated in the Northern areas, and in Sudan, primarily in the Southeast. Uganda studies focused on the Northern region, while Tanzania had sampling clusters in the Northwest (bordering Rwanda), Eastern coast, and Southern areas. Kenya's surveillance was concentrated in the Western high transmission zone near the Uganda border.

WHO other non-validated *k13* mutations distribution

A diverse landscape of other non-validated *k13* mutations was observed across East Africa, with spatial clustering of reports in the Great Lakes region and the Horn of Africa (Figure 6). Using the classification framework described in the most recent compendium of molecular markers for antimalarial drug resistance published by WHO,[27] several mutations were categorised as candidate or potential markers of artemisinin resistance. Candidate mutations showed notable regional overlap. In particular, C469F and P441L were co-reported in Rwanda, Tanzania, and Uganda, with P441L also identified in DRC and Ethiopia. Additional candidate markers included V568G, detected in Kenya and DRC, and G538V, which was reported in Zanzibar (Supplementary Table 3). Potential resistance-associated mutations were also identified across multiple countries, including F495L and Q613E in DRC, A582V in Ethiopia; A582V and N585K in Kenya, G449A in Rwanda, G625R in Sudan, Q613E in Tanzania, G625R and Y511H in Uganda, and P527H in Zanzibar (Supplementary Table 4). In addition to these variants, numerous unclassified non-synonymous mutations with unknown clinical significance were detected across the region (Supplementary Table 5). Among these, A578S, K189T and L258M were the most geographically widespread polymorphisms identified. A large number of synonymous mutations were also recorded, reflecting a high background level of sequence diversity within the *k13* propeller domain in East African *P. falciparum* populations.

Completeness of reporting and metadata gaps across studies

In addition to the identified *k13* mutations and their temporal and spatial distributions, this review identified notable gaps and inconsistencies in reporting across studies. Overall, 104 out of 126 studies (82.5%) had at least one missing key variable. A substantial proportion of studies did not report key metadata, including the lowest administrative level of sample collection (region/province: n = 14, 11.1%; town: n = 26, 20.6%), genotyping assay (n = 31, 24.6%), and participant characteristics such as age (n = 27, 21.4%). In contrast, fewer studies lacked information on the year of sample collection (n = 5, 4.0%) and sample size (n = 7, 5.6%). Notably information on where sequencing was conducted was missing in a large proportion of studies (n = 52, 41.3%). A detailed summary of reporting completeness across studies is detailed in supplementary Table 6. In some cases, information on the first-line treatment in use at the time of sampling was also absent.

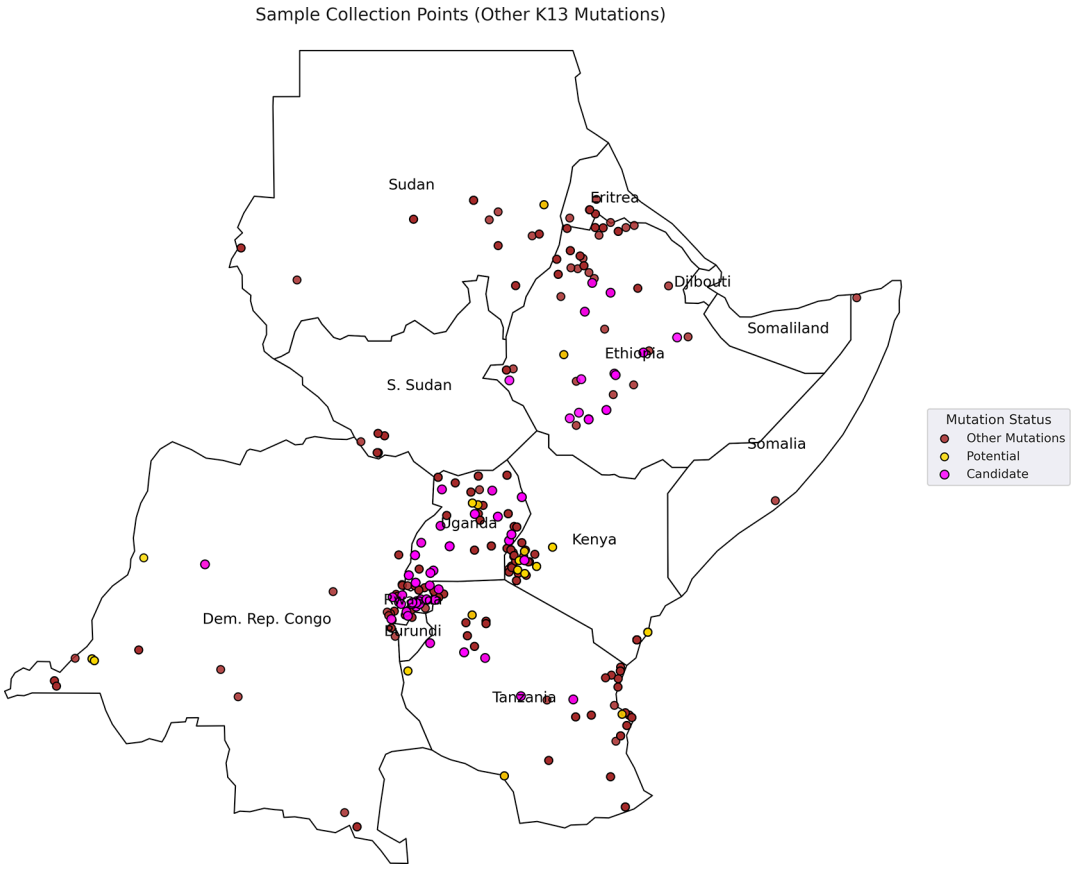


Figure 6. Geographical distribution of other (non-validated) *P. falciparum k13* mutations in East Africa. The map illustrates the spatial distribution of sample collection sites across East Africa countries where "other" candidate, potential, synonymous and non-synonymous *k13* mutations have been identified. Each round data point represents a collection location where such mutations were detected. Widespread detection of non-validated variants was observed across nearly all surveyed countries with dense clusters in Uganda, Kenya, Rwanda, and Ethiopia. Among the reported polymorphisms, A578S and K189T were the most common, followed by L258M and C469F which is a candidate marker.

Discussion

The landscape of *P. falciparum* ART-R mutations in *k13* gene is evolving across East Africa, shaped by both validated and non-validated mutations. In the chloroquine (CQ) and SP eras, resistance first emerged in Southeast (SE) Asia and later spread to Africa (Roper et al., 2003; Wootton et al., 2002). However, the detection of validated *k13* mutations in East Africa, R561H, A675V, C469Y, and R622I, different to those circulating in SE Asia, indicates an indigenous local emergence rather than importations from SE Asia.[16,19,22] Thus, once again in the malaria timeline there is a major epidemiological turning point, with East Africa as a hotspot for emerging ART-R associated mutations, reminiscent of CQ resistance emergence[4,7,28–30] and SP.[31–34] However, this time with the alert from SE Asia in 2008[35] and 2009[10] of developing clinical resistance to artemisinin and a swift identification of the genetic determinant of resistance, *k13*[14,36]; the rapid roll out of MMS[15–17,21,37] set into motion the vigilance required to serve as an early warning system for AMDR in the region.

There was heterogeneity in the MMS capacity across the region. More than 6000 samples were genotyped in the DRC, Kenya, Rwanda, Tanzania and Uganda, reflecting robust and sustained genomic surveillance programs in these countries. In contrast, Burundi, Eritrea, Somalia, South Sudan, Sudan, and Zanzibar contributed limited data, likely reflecting limited surveillance infrastructure, political instability or funding constraints. The complete absence of *k13* data from Djibouti highlights a critical surveillance gap. Such disparities create uneven regional coverage, delaying the detection of resistance focal points that could present as clinical failure, slowing down containment efforts.

The expansion of MMS activities (including *k13*) between 2014 and 2025 progressed from few samples in Ethiopia, Kenya, Rwanda, Tanzania and Uganda in the earlier years to a sharp increase in genotyped samples across several

countries in the later years. This rise likely reflects the scale-up of MMS programs and increased integration of genomic methods into national malaria control strategies. The timing also coincides with the implementation of multi-country genomic surveillance initiatives such as MalariaGEN[38,39] which have boosted genomic surveillance across Africa and the Gates Foundation funded MMS projects.[40] Despite this progress, the sparse and inconsistent sampling in several countries like Burundi, Somalia, South Sudan and Sudan, hinders the early detection of newly emerging alleles. The value of this review and molecular approaches traces the earliest detection of *k13* validated mutations to over two decades ago in East Africa (Kenya, Tanzania, Rwanda and Ethiopia), predating the recognition of clinical resistance.[17,41–43] This pattern reinforces clinical resistance following molecular emergence and underscores the importance of genomic surveillance as an essential early warning system.

The spatiotemporal increase in both the number and prevalence of validated *Pfk13* mutations in recent years suggests a regional expansion that should not go unchecked. It provides a guide to the regions to target malaria control measures. This includes countries like Ethiopia, Kenya, Rwanda, Uganda and regions emerging in Tanzania.

The regional skew in genotypes to particular geographic locations, 622I to the Horn of Africa and 469Y, 561H and 675 V to East Africa including DRC, suggests either regional gene flow or a parallel emergence under similar ecological conditions. This early indication of geographic partitioning may reflect both localized pressures such as varying ACT formulations and transmission intensities and cross border parasite flow between high malaria transmission regions.[44–46] Most data originated from high transmission border zones, including the Kenya-Uganda-Tanzania corridor,[47–51] the Rwanda-DRC border region[52–54] and specific regions like Northern Uganda.[47] These patterns suggest that the emergence of *k13* mutations in the East Africa region is a mix of local endogenous mutations rather than a single mutation sweeping through the region. However, there is evidence demonstrating the spread of mutations from Rwanda to Tanzania,[22,23] indicating the spread of some mutations in the region. Nevertheless, this is in great contrast to the situation in SE Asia, where resistance was largely driven by a single allele C580Y.[55] The alignment between sampling density and the cross-border regions that coincidentally map to the high transmission malaria regions, where the probability of resistance emergence is highest due to the high ACT pressure, supports the hypothesis of regional gene flow. However, this relationship should be interpreted with caution, as high transmission intensity can also promote genetic recombination, potentially diluting or attenuating resistance-associated mutations. These dynamics highlight the complexity of resistance emergence spread, and underscore the need for coordinated regional surveillance and containment strategies to effectively monitor and respond to transboundary AMDR.

The coexistence of multiple mutations highlights the parasite's complex evolutionary adaptability, in high transmission settings, that exhibit low linkage disequilibrium and high recombination rates. This potentially limits the generation of a single allele rising rapidly to high frequency. Of interest though, is the current absence of evidence of the historical spread previously seen of CQ and SP AMDR mutations into Africa from SE Asia rapidly changing the clinical drug resistance profile.[7,28,30,34,56]

In addition to validated *k13* mutations, 359 unique non-validated non-synonymous mutations were identified across East Africa. The most frequent variants, A578S, K189T, L258M and C469F, accounted collectively for approximately 20% of all the non-validated mutation detections. Among these, C469F has been classified as a candidate marker of artemisinin resistance in the most recent compendium of molecular markers for antimalarial drug resistance published by WHO. The widespread detection of these polymorphisms supports prior evidence that the *k13* gene exhibits extensive genetic variation in Africa.[55] These mutations are not currently linked to delayed clearance or confirmed resistance phenotypes. However, such polymorphisms may serve as evolutionary precursors that acquire adaptive function with continued ACT use.[57,58] Consequently, the continued monitoring of all *k13* mutations and whole genome sequencing of samples representing geographical spread over time are important to detect any rapid changes in mutation frequencies over time.

Despite substantial progress in MMS, several studies displayed gaps in methodologies used and the metadata presented such as, sampling location, genotyping assay, participants characteristics and first-line treatment policies. These inconsistencies weaken inter-country comparisons and meta-analyses. The adoption of standardized genotyping protocols and reporting templates would substantially enhance data quality and comparability.[59] Furthermore, establishing regional data-sharing platforms could promote real-time monitoring and policy responsiveness.

From a policy perspective, the rising prevalence of validated *k13* mutations in East Africa poses a serious threat to ACT efficacy. Although ACTs remain effective in most regions, the expansion of R561H and A675V mutations, both associated with delayed clearance, signals incipient resistance. Countries with multiple co-circulating alleles such as Tanzania, Kenya, Uganda, and Rwanda are particularly at high risk of future treatment failure. National malaria control programs should therefore integrate molecular data into treatment policy decisions, to regularly assess ACT efficacy

through therapeutic efficacy studies (TES) and explore alternatives such as triple ACTs[60] or multiple first line therapies.[61] International stakeholders, including WHO and the Africa CDC, should provide sustained technical and financial support to strengthen genomic surveillance infrastructure, ensuring early detection and rapid containment of resistance foci.

In summary, this review shows that ART-R in East Africa is no longer a hypothetical threat but an evolving reality. The region exhibits heterogeneous yet expanding *k13* mutation profiles, uneven surveillance capacity, and a worrying rise in mutation prevalence over the last 20 years. These findings place East Africa at a critical crossroad, resistance can still be contained through timely, coordinated action or left unchecked, it could mirror the catastrophic spread of chloroquine resistance three decades ago.[1,26,30,56,62] Sustained investment in standardized MMS, regional collaboration, and adaptive policy frameworks for AMDR mitigation will be crucial to preserve ACT efficacy and secure ongoing malaria control gains across the continent.

Ethics approval and consent to participate

Not applicable, as this study is based entirely on previously published data.

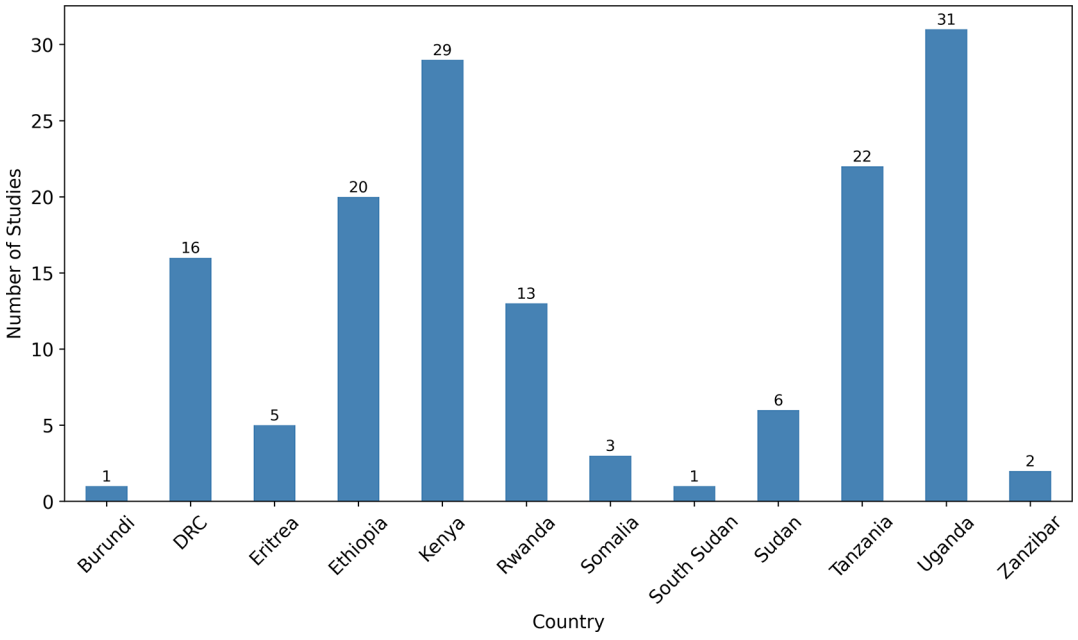
Consent for publication

All authors have read and approved the final manuscript for publication.

Authors' contributions

LIOO and DSI conceptualised and LIOO supervised the study. EA, KK and JM conducted the literature search. EA, KK, JM and KM were responsible for data acquisition, screening of primary articles, data extraction and collation. EA and KK performed the data analysis and generated the figures and tables. EA, KK, JM and KM drafted the initial manuscript. All authors (EA, KK, JM, KM, CM, HM, NA, VO, DSI, PB, and LIOO) contributed to the interpretation of findings, critically revised the manuscript, and approved the final version for submission.

Supplementary material



Supplementary Figure 1. Number of studies reporting *k13* mutations across East African countries between 2014 and 2025. Uganda (n = 31) and Kenya (n = 29) contributed the highest number of studies, followed by Tanzania (n = 22), Ethiopia (n = 20), the Democratic Republic of Congo (DRC; n = 16), and Rwanda (n = 13). Fewer studies were reported from Sudan (n = 6), Eritrea (n = 5), Somalia (n = 3), and Zanzibar (n = 2), while Burundi and South Sudan each contributed one study (n = 1). Some studies were conducted across multiple countries.

Supplementary Table 1. Summary of the number and proportion of *P. falciparum* samples analysed for *k13* mutations across East African countries between 2014 and 2025. Values are presented as counts and percentages relative to the total number of samples analysed within each country and year. The "Overall" column represents cumulative sample counts across the entire study period, while yearly columns indicate temporal distribution of surveillance efforts. Variation in sample numbers reflects differences in malaria molecular surveillance intensity, study availability, and reporting coverage across countries and years.

Country	Overall	2014	2015	2016	2017	2018	2019	2020	2021	2022	2023	2024	2025
	N = 84,024	N = 836	N = 1,247	N = 557	N = 3,106	N = 2,267	N = 2,578	N = 3,494	N = 5,623	N = 7,665	N = 15,906	N = 18,479	N = 22,266
Burundi	389 (0.46%)	0	0	0	0	0	0	0	389 (100%)	0	0	0	0
DRC	17594 (20.94%)	128 (0.73%)	0	0	580 (3.30%)	1622 (9.22%)	0	1070 (6.08%)	1684 (9.57%)	2504 (14.23%)	0	3211 (18.25%)	6795 (38.62%)
Eritrea	1237 (1.47%)	0	0	200 (16.17%)	0	0	0	144 (11.64%)	41 (3.31%)	0	852 (68.88%)	0	0
Ethiopia	3429 (4.08%)	82 (2.39%)	301 (8.78%)	0	226 (6.59%)	0	413 (12.04%)	0	0	379 (11.05%)	333 (9.71%)	649 (18.93%)	1046 (30.50%)
Kenya	14560 (17.33%)	160 (1.10%)	868 (5.96%)	136 (0.93%)	287 (1.97%)	72 (0.49%)	144 (0.99%)	551 (3.78%)	682 (4.68%)	4178 (28.70%)	3442 (23.64%)	526 (3.61%)	3514 (24.13%)
Rwanda	10615 (12.63%)	0	0	85 (0.80%)	0	0	0	954 (8.99%)	1068 (10.06%)	186 (1.75%)	2146 (20.22%)	5630 (53.04%)	546 (5.14%)
Somalia	627 (0.75%)	0	0	0	0	0	339 (54.07%)	0	138 (22.01%)	0	150 (23.92%)	0	0
South Sudan	518 (0.62%)	0	0	0	0	0	0	0	0	0	518 (100%)	0	0
Sudan	437 (0.52%)	0	0	0	0	0	31 (7.09%)	186 (42.56%)	0	0	92 (21.05%)	0	128 (29.29%)
Tanzania	23611 (28.10%)	142 (0.60%)	0	36 (0.15%)	0	324 (1.37%)	87 (0.37%)	589 (2.49%)	652 (2.76%)	0	7782 (32.96%)	6788 (28.75%)	7211 (30.54%)
Uganda	9056 (10.78%)	324 (3.58%)	78 (0.86%)	100 (1.10%)	2013 (22.23%)	249 (2.75%)	1564 (17.27%)	0	969 (10.70%)	418 (4.62%)	73 (0.81%)	1675 (18.50%)	1593 (17.59%)
Zanzibar	1951 (2.32%)	0	0	0	0	0	0	0	0	0	518 (26.55%)	0	1433 (73.45%)

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Supplementary Table 2. Distribution and diversity of WHO-validated *k13* mutations identified across ten East African countries between 2014 and 2025. The table summarizes the number of unique validated mutations detected per country and lists the corresponding mutations according to current WHO classification criteria. Tanzania exhibited the highest diversity of validated mutations,[6] followed by Kenya,[5] while several countries showed overlapping mutation profiles. C580Y was exclusively reported in Ethiopia, whereas R622I was predominantly observed in Ethiopia, Eritrea, Somalia, and Sudan.

Country	Number of unique mutations	Validated mutations
Tanzania	6	C469Y, M476I, R561H, P574L, R622I, A675V
Kenya	5	C469Y, R539T, P553L, R561H, A675V
Ethiopia	4	P574L, C580Y, A675V, R622I
Rwanda	4	C469Y, R561H, P574L, A675V
Uganda	4	C469Y, R561H, P574L, A675V,
Sudan	3	C469Y, R561H, R622I
DRC	3	C469Y, R561H, A675V
Eritrea	2	R561H, R622I
Somalia	1	R622I
Zanzibar	1	R561H

Supplementary Table 3. Distribution of WHO candidate *k13* mutations detected across East African countries. The table presents the number and types of candidate mutations detected per country according to current WHO classification criteria. P441L and C469F were the most frequently reported candidate mutations, occurring across multiple countries.

Country	Number of mutations	Candidate mutations
DRC	2	P441L, V568G
Rwanda	2	C469F, P441L
Tanzania	2	C469F, P441L
Uganda	2	C469F, P441L
Kenya	1	V568G
Zanzibar	1	G538V
Ethiopia	1	P441L

Supplementary Table 4. Spatial profile of WHO-classified potential marker *P. falciparum k13* mutations detected across East African countries over the study period.

Country	Number of mutations	Potential mutations
DRC	2	F495L, Q613E
Kenya	2	A582V, N585K
Uganda	2	G625R, Y511H
Ethiopia	1	A582V
Rwanda	1	G449A
Sudan	1	G625R
Tanzania	1	Q613E
Zanzibar	1	P527H

Supplementary Table 5. Country-wise distribution of other non-validated synonymous and non-synonymous *k13* mutations reported.

Country	Number of mutations	Other mutations
DRC	27	A557S, A578S, A617T, C532S, D516A, D584E, E509D, G496G, K189T, M472L, M476K, N523T, P417P, C469C, R471R, S477S, T478T, G496G, Y511Y, R539R, S576S, S477Y, R255K, R471R, R513R, S477Y, S522C, S576T, V520A, V568M, V581V, V589V, V637A, V637I, Y493Y, Y588C
Eritrea	6	D281V, E208K, E401Q, K189N, K189T, T535M
Ethiopia	18	A504T, D281V, E208K, E401Q, E433D, H136N, K189N, K189T, M18T, N135H, N531I, N599I, R255K, R716I, S550F, T535A, V714D, Y630F
Kenya	112	“504, 510”, A174S, A486S, A504G, A554S, A569S, A569T, A578S, A671V, A676V, C439C, C469C, C469T, C469W, C542R, D399N, D584Y, E507E, E509E, E509G, E596R, E612D, E620K, E643E, F451Y, G533G, G538G, G548G, G592E, G665S, G690G, H136N, H384Y, H560H, I134S, I148T, I178L, I354V, I354V, A486S, V487A, S522C, A578S, A582V, S600A, I634L, V666L, A676S, I416V, I448M, I590F, I646L, K108E, K189T, L119L, L258M, L457I, L505F, L598F, L678V, M157V, M442V, N431S, N458Y, N490S, N498I, N530I, N530K, N530Y, N531I, N531N, N554S, N587K, N609L, Nx6–137-Nx10, Nx6–137-Nx7, Nx6–137-Nx8, Nx6–137-Nx9, P417P, P553P, P570L, P655P, P667S, P96Q, Q271H, R255K, R513S, R529K, R539K, R539T, R597R, R622G, S182T, S38C, S477S, S485K, S522C, S522R, S536L, S549P, T126N, T149S, T192A, T474T, T478T, T535T, T677A, T677K, T677R, T677S, V487V, V520A, V568I, V589V, V637V, W518C, W611S, Y328F, Y500Y, Y616N, Y630F
Rwanda	30	555A, 578S, A569V, A578S, A578V, A626E, A626S, C532W, D247Y, D648H, E605K, E651K, F699C, G533A, G592E, G592E + V637I, I205T, K189T, M460I, N133K, N217H, P667R, P667S, Q661E, R513L, R575I, R575K, V487I, V555A, V637I
South Sudan	15	A486S, A578S, D516D, E567E, E602K, E605K, F673F, G449G, G496G, N694N, P615S, Q654Q, R561L, S623G, Y519H
Sudan	34	A504T, A578S, A621V, A626S, A626T, C542Y, D389H, D389N, E401Q, E433D, E461Q, E509E, E606K, E620G, F375S, F442F, K372K, K378R, K430E, K430K, L488L, N594K, P443P, Q661P, Q661R, R561H, R575T, S600F, T667I, V494I, V589I, W565STOP, Y541H, Y635C
Tanzania	67	A175T, A359T, A427S, A578S, A676T, A724P, C469C, C473C, E208K, E433D, E556K, E602D, F699C, G112E, G496S, G497D, G538G, G591E, G690D, H136N, H384R, I205T, I352T, I354V, I416V, I526I, K108E, K124N, K189N, K189T, K9R, L116I, L177I, L258M, L463S, L463S, G496S, M476I, V510M, E556K,M562T, E602D, L99V, M562T, N133K, N137D, N137K, N217H, N264S, N689S, P417P, P419S, P443S, P475S, P667S, P667T, Q271L, Q661E, R255K, R471S, S182T, S459, S576L, S624S, T149S, T207K, T348I, V487V, V510M, V520A, V520I, V555A, Y493Y
Uganda	89	A178T, A504A, A569S, A569T, A569V, A578S, A617T, A621S, A724P, C1801G, C469C, C532S, E461G, E509E, E567K, E596V, E620K, E643K, F334L, F439F, F442L, F491S, F699C, G1929A, G496S, G533A, G533C, G533G, G595C, G638R, G718S, H136N, I448V, I465T, I540T, I587I, I646I, K189N, K189T, K563E, K607E, K610E, L189T, L258M, L368L, L457I, L618S, L619S, L631F, L708I, L713F, M472V, N490T, N594K, P441A, P475S, P615N, P667L, P667S, Q467Q, Q654R, R539T, R561R, R575K, S522C, S522R, S522S, S600C, S695T, T348I, T49S, T685P, V517I, V517V, V555A, V581A, V581I, V637D, V637I, V650I, V661I, V666I, W470R, W565R, Y546H, Y558C, Y558H, Y588N, Y630F
Zanzibar	2	A481V, N573I

Supplementary Table 6. Completeness of metadata reporting across included studies. This table summarizes the completeness of key metadata fields extracted from studies included in the analysis. For each variable, the number and proportion of studies with missing data are presented. Missing information was most common for sequencing location and genotyping assay, indicating substantial heterogeneity and gaps in reporting across studies. These inconsistencies limit cross-study comparability and underscore the need for standardized reporting frameworks in malaria molecular surveillance.

variable	n_missing	percent_missing
year_sample	5	4
region/province	14	11.1
town	26	20.6
participants_age	27	21.4
sample_size	7	5.6
genotyping_assay	31	24.6
study_population	7	5.6
samples_genotyped	4	3.2
where_sequenced	52	41.3
other_k13_mutations	39	31

Availability of data and materials

All data supporting the findings of this study are provided within the manuscript and its supplementary information files.

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