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REVIEW

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# Prevalence of antimalaria drug resistance-conferring mutations associated with sulphadoxine-pyrimethamineine-resistant *Plasmodium falciparum* in East Africa: a systematic review and meta-analysis

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## Abstract

**Background** The emergence and spread of drug resistance to antimalarial drugs pose a severe threat to effective malaria control and treatment. Although sulfadoxine-pyrimethamine resistance is well-documented, it is still the drug of choice for treating intermittent resistance. Molecular markers play a crucial role in tracking and understanding the prevalence of antimalarial drug resistance. Currently, there is insufficient information on the prevalence of molecular markers associated with sulfadoxine-pyrimethamine resistance in *P. falciparum*.

**Objective** This systematic review and meta-analysis aimed to determine the pooled prevalence of antimalaria drug resistance-conferring markers associated with sulphadoxine-pyrimethamineine in *Plasmodium falciparum* in East Africa.

**Methods** Systematic searche was performed to retrieve articles from PubMed, Scopus, Science Direct databases, and Google Scholar search engine. Sixteen potential studies that provided important data on markers for sulphadoxine-pyrimethamineine resistance in *Plasmodium falciparum* were systematically reviewed and analyzed. Nine antimalarial drug resistance markers responsible for sulphadoxine-pyrimethamineine resistance in *Plasmodium falciparum* were extracted separately into Microsoft Excel and analyzed using STATA 17.0. The inverse of variance was done to evaluate heterogeneity across studies. A funnel plot was used to determine the presence of publication bias. A trim-and-fill-meta-analysis was carried out to generate a bias-adjusted effect estimate. A random effect model was used to determine the pooled prevalence of markers responsible for sulphadoxine-pyrimethamineine resistance. Subgroup analysis was performed based on country and year of publication.

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**Results** A total of 16 studies were included for this systematic review and meta-analysis.The molecular markers like dhfr (N51I, C59R, S108N, 108N, 59R, and I164L), and dhps (A437G, K540E, & 540E) were selected for meta-analysis. From this meta-analysis, the pooled prevalence of dhfr N51I, dhfr C59R, dhfr S108N, dhfr 108N, dhfr 59R, and dhfr I164L was 88.6%, 85.3%, 89.6%, 92.2%, 71.5%, and 3.9%, respectively. Likewise, the aggregated prevalence of dhps A437G, dhps K540E, and dhps 540E was 90.2%, 80.9%, and 91.5%, respectively. The subgroup analysis based on year of publication showed that the pooled prevalence of dhfr N51I, dhfr C59R, dhfr S108N, dhps A437G, and dhps K540E, in studies conducted 2014–2018 was 97.11%, 90.57%, 96.45%, 90.89%, and 89.45%, respectively, while it was 82.03%, 81.78%, 85.12%, 89.24%, and 73.98%, respectively, in studies conducted 2019–2023. On the other hand, country-based analysis showed that the pooled prevalence of dhfr N51I, dhfr C59R, dhfr S108N, dhps A437G, and dhps K540E, in Kenya was 85.88%, 84.02%, 86.56%, 90.7%, and 77.55%, respectively.

**Conclusions** This systematic review and meta-analysis reveal a high prevalence of drug resistance markers associated with sulphadoxine-pyrimethamine resistance in *Plasmodium falciparum* across the East African region. This underscores the significant challenges in managing malaria infections caused by *Plasmodium falciparum* in the region. Therefore, regular monitoring, identification, and limiting of drug-resistance markers and drug-resistant *P. falciparum* strains must be sustained to ensure the effectiveness of malaria treatment.

**Keywords** Prevalence, *Plasmodium falciparum*, Drug resistance, Marker, Sulphadoxine-pyrimethamineine, East Africa

## Introduction

Malaria is a mosquito-borne infectious disease of humans and animals, which is caused by a protozoan parasite of the genus *Plasmodium* [1]. More than 100 species of *Plasmodium* can infect numerous animals, but only four species of parasite can infect humans, such as *Plasmodium falciparum, Plasmodium vivax, Plasmodium ovale,* and *Plasmodium malariae* [2]. However, *P. falciparum* is the most common and detrimental malaria parasite, accounting for 99.7% of malaria cases, and it frequently causes severe disease and death, particularly in the World Health Organization African region [3, 4]. In 2020, around 241 million cases and 627 thousand deaths of malaria were estimated worldwide [5]. *P. falciparum* infection is the cause of over 90% of malaria deaths worldwide, making it a persistent danger to public health on a global scale [6]. Over 200 million clinical cases and over 400,000 fatalities in Africa are caused by *P. falciparum* infection each year, accounting for 92% of the malaria burden worldwide [7].

De novo drug resistance to malaria must arise from spontaneous mutations or gene duplications that confer reduced drug susceptibility [8]. These mutations or duplications are selected in individuals when antimalarial drug concentrations are high enough to kill or inhibit susceptible parasites while allowing the resistant clones to thrive [9]. The emergence and spread of drug resistance to commonly used antimalarial drugs pose a severe threat to effective malaria control and treatment [10]. Over the years, the effectiveness of various antimalarial drugs has been compromised by the emergence and spread of drug-resistant malaria parasites [11]. Antimalarial resistance in non-falciparum species has developed more slowly, possibly due to fewer genetic mutations and lower parasite loads in the human host; for instance, in *P. vivax* and *P. ovale*, this slower development is likely related to their ability to evade blood schizonticides by forming hypnozoites in the liver [12]. The worldwide efforts against malaria are facing significant obstacles due to the increasing prevalence of *P. falciparum* resistance to vital anti-malarial drugs [13]. Drug-resistant *P. falciparum* caused a disastrous rise in sub-Saharan African countries, where case incidence and mortality doubled or tripled [14]. Drug-resistant *P. falciparum* is still found in a variety of locations around the world today, partly due to trends in drug delivery and the intensity of transmission [15].

Despite well-documented resistance to sulfadoxine-pyrimethamine (SP), it remains the drug of choice for treating intermittent malaria and assessing resistance levels to inform national policy decisions [16]. Genetic changes (mutations) in the *P. falciparum* dihydrofolate reductase (*pfdhfr*) and dihydropteroate synthetase (*pfdhps*) genes confer SP resistance [17]. The pfdhps A437G codon, when combined with the pfdhfr triple mutant/codon change at N51I, C59R, and S108 N allele, has been linked to treatment failure [18]. In East Africa, a quintuple mutant genotype with the pfdhfr triple mutant and the pfdhps double (A437G + K540E) mutations is a significant predictor of SP treatment failure [19].

Molecular markers play a crucial role in tracking and understanding the prevalence of antimalarial drug resistance, monitoring the efficacy of existing antimalarial treatments, and guiding the development of new therapeutic strategies [20, 21]. These markers are specific genetic variations or mutations within the parasite's genome that confer resistance to antimalarial

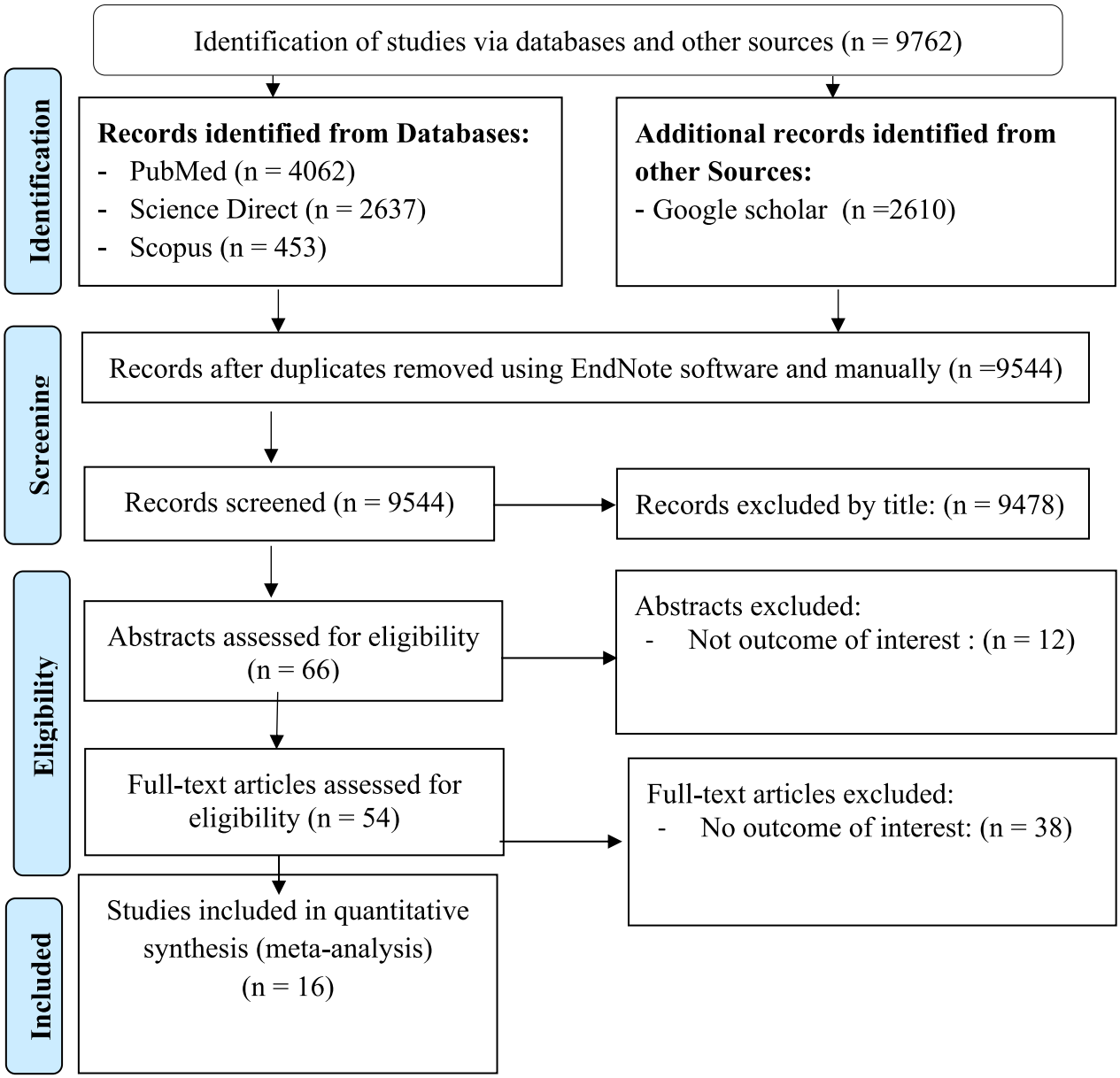


**Fig. 1** PRISMA flow diagram indicated the results of the search and reasons for exclusion [32]

drugs [22]. By analyzing these molecular markers, scientists can identify and monitor the spread of drug-resistant malaria parasites in different regions [23]. The prevalence of molecular markers of antimalarial drug resistance varies across different geographical areas and parasite species [24]. For instance, in some regions, there is a high prevalence of molecular markers associated with resistance to artemisinin-based combination therapies, the current frontline treatment for malaria [25]. Besides, the prevalence of molecular markers of anti-malaria drug resistance is a critical issue in the field of malaria research and public health [26]. This highlights the urgent need for continued surveillance and research to develop new antimalarial drugs and strategies to combat drug resistance [27].

Researchers can determine the degree of drug resistance and adjust treatment regimens by studying the frequency of particular molecular markers, such as *Pfdhfr* and *Pfdhps*, among malaria parasite populations in different regions [28]. This information is crucial for informing national malaria control programs, guiding the selection of appropriate antimalarial drugs, and preventing the

**Table 1** Summary of included studies in systematic review and meta-analysis

| Author | Year of publication | Country | study design | Gene | Marker | Number of sample sequenced (N) | prevalence of mutation (N/%) | Quality score/9 |
|---|---|---|---|---|---|---|---|---|
| Gabriel et al. [33] | 2019 | Kenya | Cohort study | Pfdhfr | N51I | 76 | 71/93.4 | 9 |
| | | | | | S108 N | 76 | 70/92 | |
| | | | | Pfdhps | A437G | 76 | 71/93.4 | |
| | | | | | K540E | 76 | 69/90.8 | |
| Fredrick et al. [48] | 2015 | Rewanda | Cross sectional study | Pfdhfr | N51I | 376 | 375/99.7 | 8 |
| | | | | | C59R | 381 | 344/90.3 | |
| | | | | | S108 N | 379 | 379/100 | |
| | | | | | I164L | 377 | 19/5 | |
| | | | | | Quadruple (N51I-C59R-S108 N-I164L) | 399 | 18/4.5 | |
| | | | | | Triple (N51I-C59R-S108 N) | 399 | 339/85 | |
| | | | | Pfdhps | A437G | 364 | 338/93 | |
| | | | | | K540E | 363 | 343/94 | |
| | | | | | Double (A437G-K540E) | 399 | 334/83.7 | |
| | | | | | Triple (A437G-K540E-A581G) | 402 | 339/84.3 | |
| Paulo et al. [35] | 2019 | Mozambique | Cross sectional study | Pfdhfr | N51I | 100 | 89/89 | 9 |
| | | | | | C59R | 100 | 86/86 | |
| | | | | | S108 N | 100 | 90/90 | |
| | | | | | I164L | 100 | 2/2 | |
| | | | | | Triple [N51I-C59R-S108 N] | 100 | 90/90 | |
| | | | | Pfdhps | A437G | 100 | 74/74 | |
| | | | | | K540E | 100 | 79/79 | |
| | | | | | Double (A437-K540E) | 100 | 72/72 | |
| | | | | | Triple [A437G-K540E-A581G] | 14 | 11/79 | |
| Vito et al. [43] | 2015 | Tanzania | Cross sectional study | Pfdhfr | CIRNI | 264 | 186/70.4 | 9 |
| | | | | | CICNI | 264 | 28/10.6 | |
| | | | | | CNRNI | 264 | 16/6 | |
| | | | | | S108 N | 264 | 244/92.4 | |
| | | | | | C59R | 264 | 221/83.7 | |
| | | | | Pfdhps | AGEAA | 264 | 96/36.4 | |
| | | | | | SGEAA | 264 | 36/13.6 | |
| | | | | | SGEGA | 264 | 33/12.5 | |
| Zhiyong et al. [28] | 2022 | Kenya | Community based household surveys | Pfdhfr | N51I | 323 | 323/100 | 9 |
| | | | | | S108 N | 346 | 323/93 | |
| | | | | | C59R | 346 | 323/93 | |
| | | | | Pfdhps | A437G | 346 | 323/93 | |
| | | | | | K540E | 346 | 319/92 | |
| Ashley et al. [47] | 2023 | Kenya | NA | Pfdhfr | N51I | 53 | 41/77 | 7 |

**Table 1** (continued)

| Author | Year of publication | Country | study design | Gene | Marker | Number of sample sequenced (N) | prevalence of mutation (N/%) | Quality score/9 |
|---|---|---|---|---|---|---|---|---|
| | | | | | C59R | 52 | 40/77 | |
| | | | | | S108 N | 52 | 42/81 | |
| | | | | | I164L | 52 | 3/6 | |
| | | | | | Quintuple (N51I-C59R- S108 N/A437G-K540E) | 106 | 44/41.5 | |
| | | | | Pfdhps | K540E | 48 | 38/79 | |
| | | | | | A437G | 42 | 42/100 | |
| Irene et al. [46] | 2023 | South Sudan | Longitudinal time series | Pfdhfr | 51I | 532 | 517/97 | 8 |
| | | | | | 108 N | 532 | 516/97 | |
| | | | | | 59R | 532 | 447/84 | |
| | | | | | IRNGE | 532 | 332/62 | |
| | | | | | 581G | 532 | 34/6.4 | |
| | | | | | 164L | 532 | 4/0.8 | |
| | | | | Pfdhps | 437G | 532 | 435/82 | |
| | | | | | 540E | 532 | 460/86.5 | |
| Monica et al. [41] | 2015 | Kenya | Cross sectional study | Pfdhfr | Triple (51I/59R/108 N) | 253 | 223/88 | 9 |
| | | | | dhfr/dhps | quintuple (dhfr 51I/59R/108 N + dhps 437G/540E) | 253 | 215/85 | |
| | | | | Pfdhps | Double (437G/540E) | 253 | 243/96 | |
| Melissa et al. [39] | 2017 | Kenya | Clinical trial study | Pfdhfr | N51I | 120 | 115/95.8 | 8 |
| | | | | | C59R | 121 | 117/96.7 | |
| | | | | | S108 N | 121 | 117/96.7 | |
| | | | | Pfdhps | A437G | 50 | 48/96 | |
| | | | | | K540E | 113 | 103/91 | |
| Stella et al. [45] | 2020 | Kenya | Cross sectional study | Pfdhfr | quintuple (51I + 59R + 108 N) + (437G + 540E) | 106 | 91/85.8 | 9 |
| | | | | | 51I | 106 | 94/88.7 | |
| | | | | | 59R | 106 | 83/78 | |
| | | | | | 108 N | 106 | 99/93.4 | |
| | | | | | Quadruple [(51I + 59R + 108 N) + 437G] | 106 | 11/10.4 | |
| | | | | | Quadruple [(59R + 108 N) + (437G + 540E)] | 106 | 11/10.4 | |
| | | | | | Triple (51I + 59R + 108 N) | 106 | 4/37.7 | |
| | | | | | 51I | 106 | 94/88.7 | |
| | | | | Pfdhps | 437G | 106 | 100/94 | |
| | | | | | 540E | 106 | 97/91.5 | |
| Anthony et al. [40] | 2015 | Uganda | Molecular epidemiological study | Pfdhfr | 108 N | 120 | 120/100 | 9 |
| | | | | | 59R | 116 | 112/96.6 | |
| | | | | | 51I | 111 | 111/100 | |

drug-resistant malaria parasite strains [27]. Exploring the prevalence of molecular markers of

**Table 1** (continued)

| Author | Year of publication | Country | study design | Gene | Marker | Number of sample sequenced (N) | prevalence of mutation (N/%) | Quality score/9 |
|---|---|---|---|---|---|---|---|---|
| | | | | | double (51I-108 N) | 111 | 111/100 | |
| | | | | | Double (59R-108 N) | 116 | 112/96.6 | |
| | | | | | triple (51I-59R-108 N) | 110 | 107/97.3 | |
| | | | | | Quadruple (51I- 59R- 108 N) −437G | 110 | 107/97.3 | |
| | | | | | Quintuple (51I −59R-108 N) (437G −540E) | 110 | 106/96.4 | |
| | | | | | Sextuple (51I-59R- 108 N) (437G −540E −581G) | 101 | 8/7.9 | |
| | | | | Pfdhps | 581G | 103 | 8/7.8 | |
| | | | | | 437G | 120 | 120/100 | |
| | | | | | 540E | 120 | 119/99 | |
| | | | | | double (437G-540E) | 120 | 119/99 | |
| Mwiche et al. [42] | 2015 | Zambia | Cross sectional study | Pfdhfr | triple ((Asn-108 + Ile-51 + Arg-59) | 72 | 49/68 | 9 |
| | | | | Pfdhps | Double (Gly −437 + Glu-540) | 72 | 15/20.8 | |
| Eugenia et al. [38] | 2017 | Ethiopia | Cross sectional study | Pfdhfr | 51I | 199 | 154/77.4 | 9 |
| | | | | | 59R | 199 | 99/49.7 | |
| | | | | | 108 N | 199 | 155/77.9 | |
| | | | | Pfdhps | 396 K | 199 | 72/36 | |
| Dennis et al. [37] | 2019 | Kenya | Unblinded, randomized controlled trial | Pfdhfr | N51I | 101 | 61/60.4 | 9 |
| | | | | | C59R | 101 | 55/54.5 | |
| | | | | | S108 N | 101 | 61/60.4 | |
| | | | | | I164L | 101 | 4/4 | |
| | | | | Pfdhps | A437G | 101 | 64/63.4 | |
| | | | | | K540E | 101 | 66/65 | |
| | | | | | A581G | 101 | 1/1 | |
| Reginald et al. [44] | 2016 | Tanzania | | Pfdhps | K540E | 928 | 821/88.5 | 8 |
| | | | | | A581G | 854 | 150/17.6 | |
| Himanshu et al. [34] | 2018 | Mozambique | Descriptive observational study | Pfdhps | S436 F | 345 | 10/2.8 | 9 |
| | | | | | A437G | 345 | 289/83.8 | |
| | | | | | K540E | 348 | 286/82 | |

NA = Non-applicable

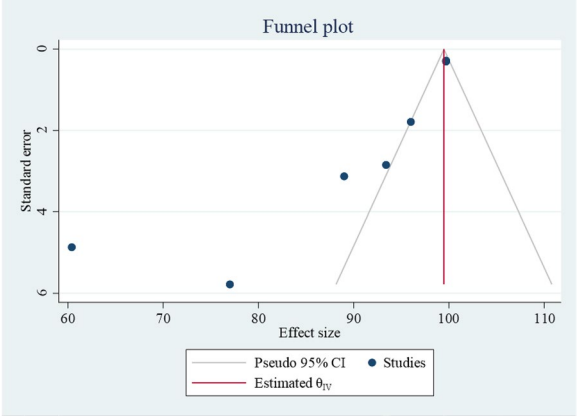


**Fig. 2** Funnel plot for prevalence of dhfr N51I mutation

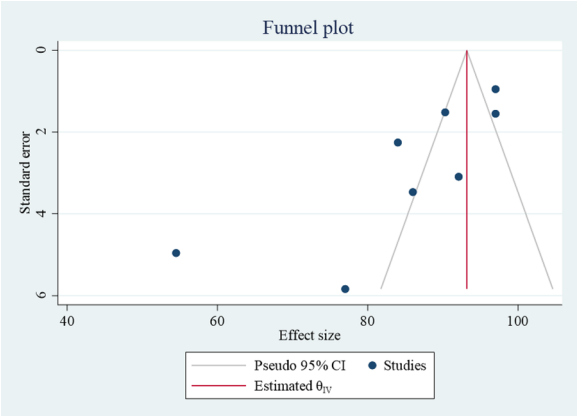


**Fig. 3** Funnel plot for prevalence of dhfr C59R mutation

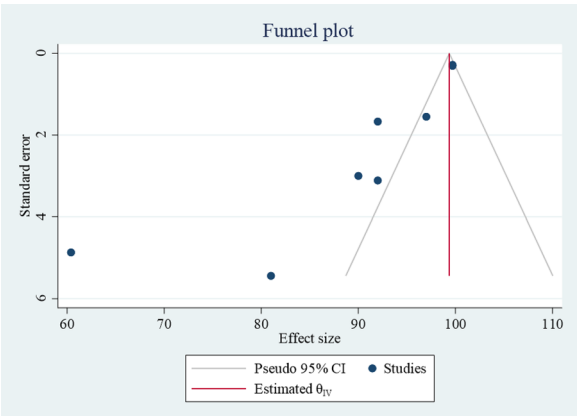


**Fig. 4** Funnel plot for prevalence of dhfr S108 N mutation

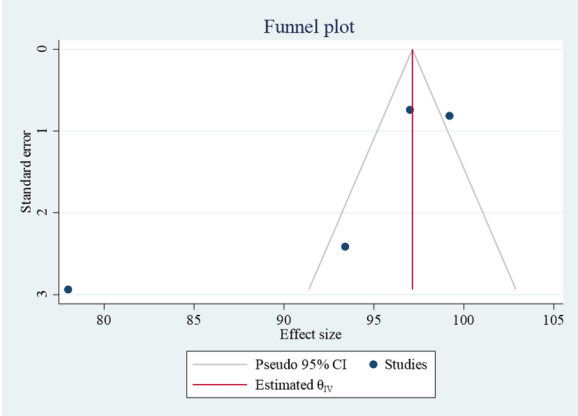


**Fig. 5** Funnel plot for prevalence of dhfr 108 N mutation

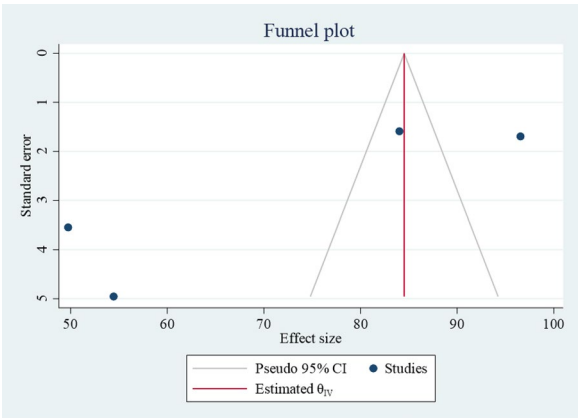


**Fig. 6** Funnel plot for prevalence of dhfr 59R mutation

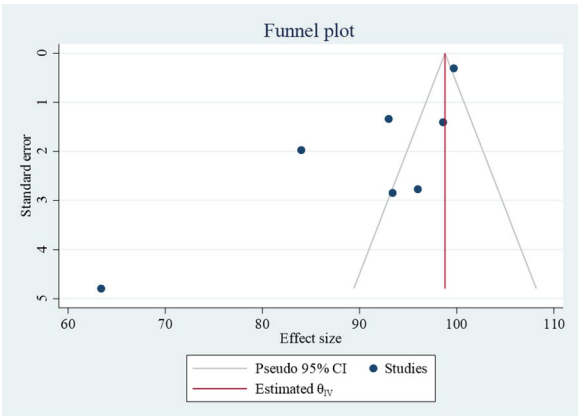


**Fig. 7** Funnel plot for prevalence of dhps A437G mutation

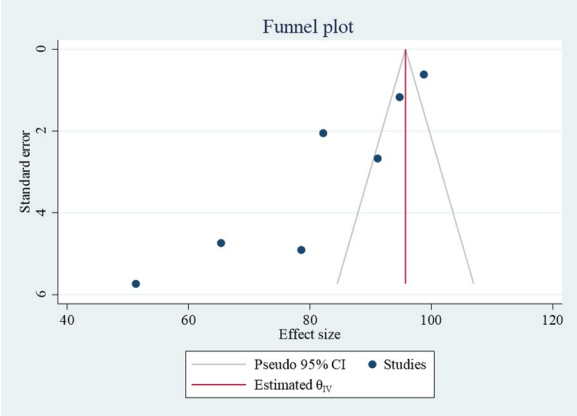


**Fig. 8** Funnel plot for prevalence of dhps K540E mutation

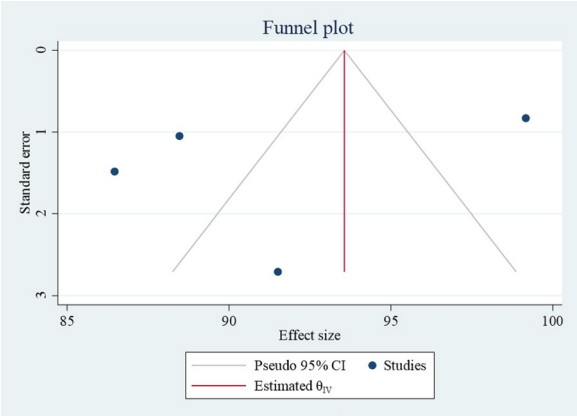


**Fig. 9** Funnel plot for prevalence of dhps 540E mutation

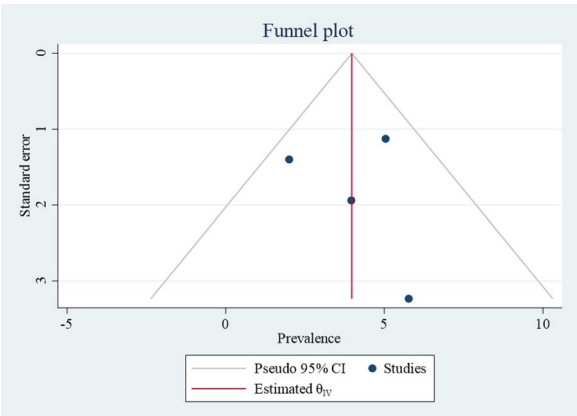


**Fig. 10** Funnel plot for prevalence of dhfr I164L mutation

**Table 2** Trim-and-fill analysis for the prevalence of dhfrN51I mutation

| Studies | Effect size | [95% CI] |
|---|---|---|
| Observed (7) | 88.55 | 78.2–98.87 |
| Observed + Imputed (7 + 0) | 88.55 | 78.2–98.87 |

**Table 3** Trim-and-fill analysis for the prevalence of dhfr C59R mutation

| Studies | Effect size | [95%CI] |
|---|---|---|
| Observed (8) | 85.3 | 76.1–94.59 |
| Observed + Imputed (8 + 2) | 81.1 | 71.98–90.2 |

**Table 4** Trim-and-fill analysis for the prevalence of dhfr S108 N mutation

| Studies | Effect size | [95%CI] |
|---|---|---|
| Observed (8) | 89.6 | 81.1–98.2 |
| Observed + Imputed (8 + 2) | 85.9 | 77.65–94.2 |

**Table 5** Trim-and-fill analysis for the prevalence of dhfr 108 N mutation

| Studies | Effect size | [95%CI] |
|---|---|---|
| Observed (4) | 92.2 | 83.1–101.3 |
| Observed + Imputed (4 + 1) | 89.7 | 81.1 –98.3 |

**Table 6** Trim-and-fill analysis for the prevalence of dhfr 59R mutation

| Studies | Effect size | [95%CI] |
|---|---|---|
| Observed (4) | 71.5 | 49.2–93.77 |
| Observed + Imputed (0) | 71.5 | 49.2–93.77 |

**Table 7** Trim-and-fill analysis for the prevalence of dhps A437G mutation

| Studies | Effect size | [95%CI] |
|---|---|---|
| Observed (7) | 90.2 | 81.46–98.98 |
| Observed + Imputed (7 + 2) | 86.39 | 78.1–94.7 |

anti-malaria drug resistance provides valuable insights into the dynamics of drug resistance in malaria parasites and underscores the importance of continuous

**Table 8** Trim-and-fill analysis for the prevalence of dhps K540E mutation

| Studies | Effect size | [95%CI] |
|---|---|---|
| Observed (7) | 80.9 | 68.6–93.2 |
| Observed + Imputed (7 + 0) | 80.9 | 68.6–3.2 |

**Table 9** Trim-and-fill analysis for the prevalence of dhps 540E mutation

| Studies | Effect size | [95%CI] |
|---|---|---|
| Observed (4) | 91.5 | 85.7–97.2 |
| Observed + Imputed (4 + 0) | 91.5 | 85.7–97.2 |

**Table 10** Trim-and-fill analysis for the prevalence of dhfr I164L mutation

| Studies | Effect size | [95%CI] |
|---|---|---|
| Observed (4) | 3.9 | 2.1–5.77 |
| Observed + Imputed (4 + 1) | 3.8 | 2.1–5.51 |

surveillance and research efforts to combat this significant public health challenge [29].

Currently, there is insufficient information on the prevalence of molecular markers for SP-resistant *P. falciparum* and their implications for anti-malarial policies. This makes it more difficult to compare resistance patterns throughout the study area and to coordinate efforts to address the problem of drug resistance globally. We present a comprehensive study utilizing systematically extracted data from English-published and unpublished articles conducted over the past decade across East African countries. Therefore, this systematic review and meta-analysis aimed to determine the pooled prevalence of genetic changes responsible for the antimalaria drug, sulfadoxine-pyrimethamine resistance in *P. falciparum* in East African countries from 2014 to 2023.

## Methods

### Review protocol

We followed the Preferred Reporting Items for Systematic Review and Meta-analyses (PRISMA) guidelines to search articles from online databases, literature screening by title and abstract, and assess the full-text's appropriateness. The review protocol was developed before literature searching and was registered with the International Prospective Register of Systematic Reviews (PROSPERO) database with registration number CRD42024580210.

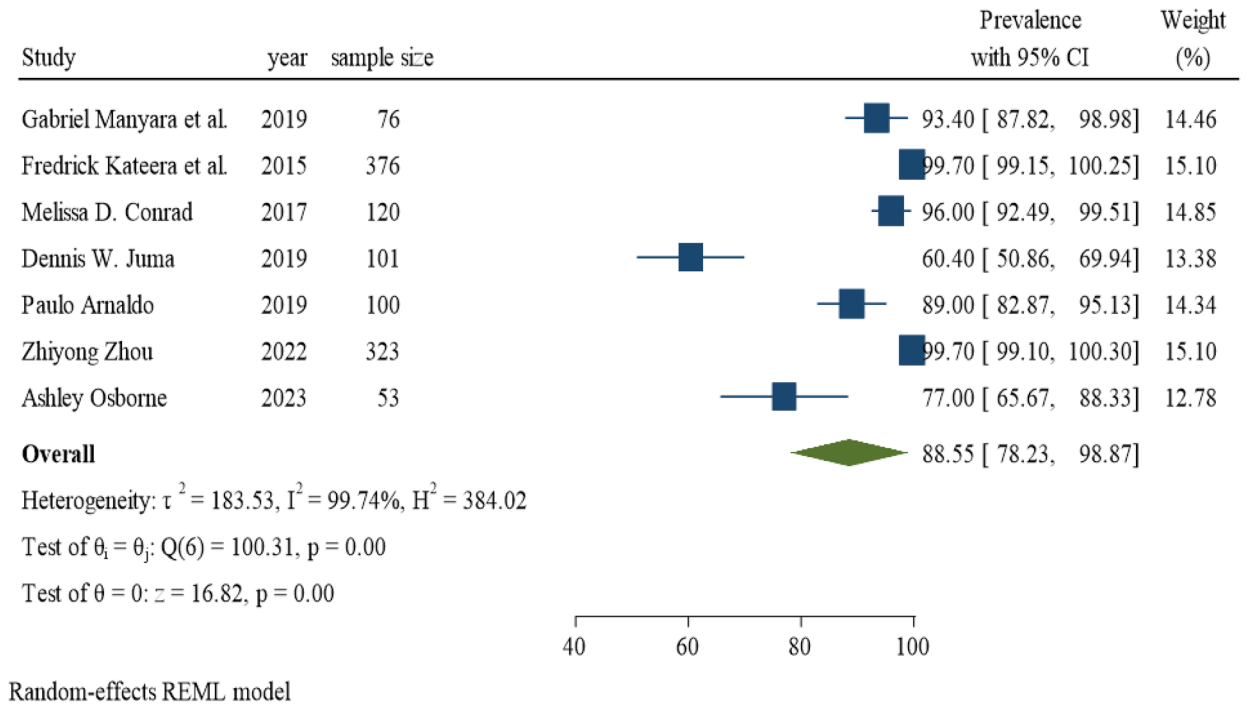


**Fig. 11** Forest plot showing the prevalence of dhfr N51I mutation

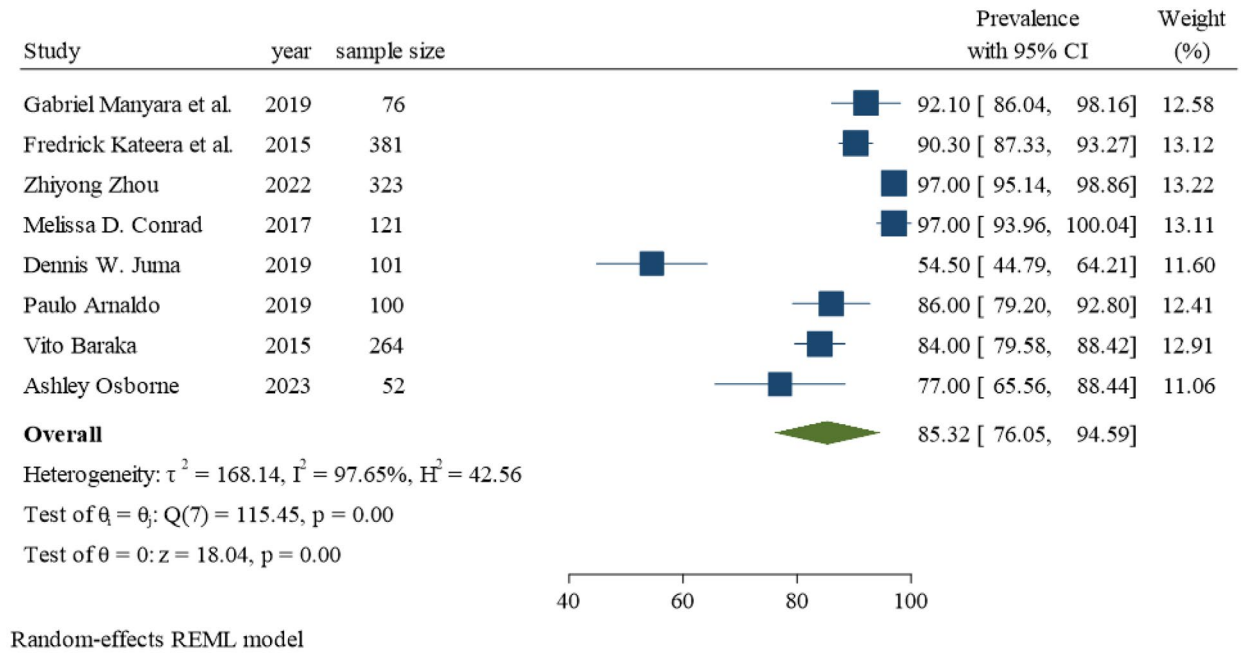


**Fig. 12** Forest plot showing the prevalence of dhfr C59R mutation

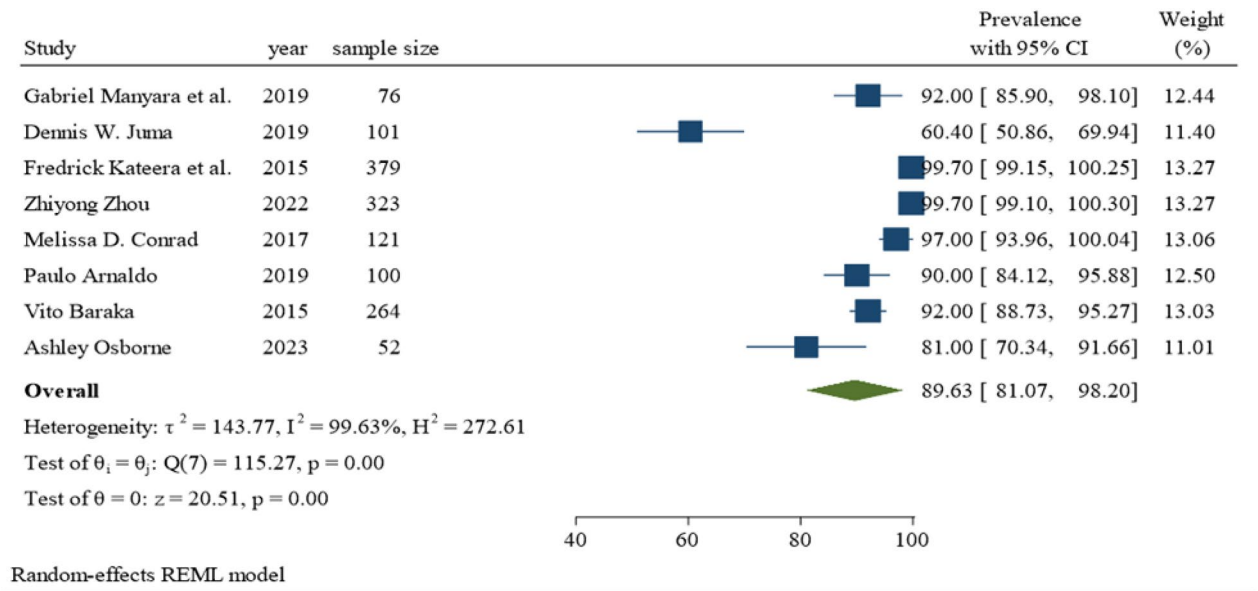


**Fig. 13** Forest plot showing the prevalence of dhfr S108 N mutation

### Data management

The data for the review articles was managed using EndNote software version X7 (Thomson Reuters, 2015). The software imported all detected article titles and removed duplicates. Then, using specified criteria, article titles were filtered and classified into several eligibility groups (included or excluded). The Excel 2010 data extraction form was pretested on five articles and then changed depending on the pilot test results.

### Search strategy

A systematic search strategy, utilizing a combination of keywords, was implemented to search for articles in PubMed, ScienceDirect, Scopus, and the Google Scholar

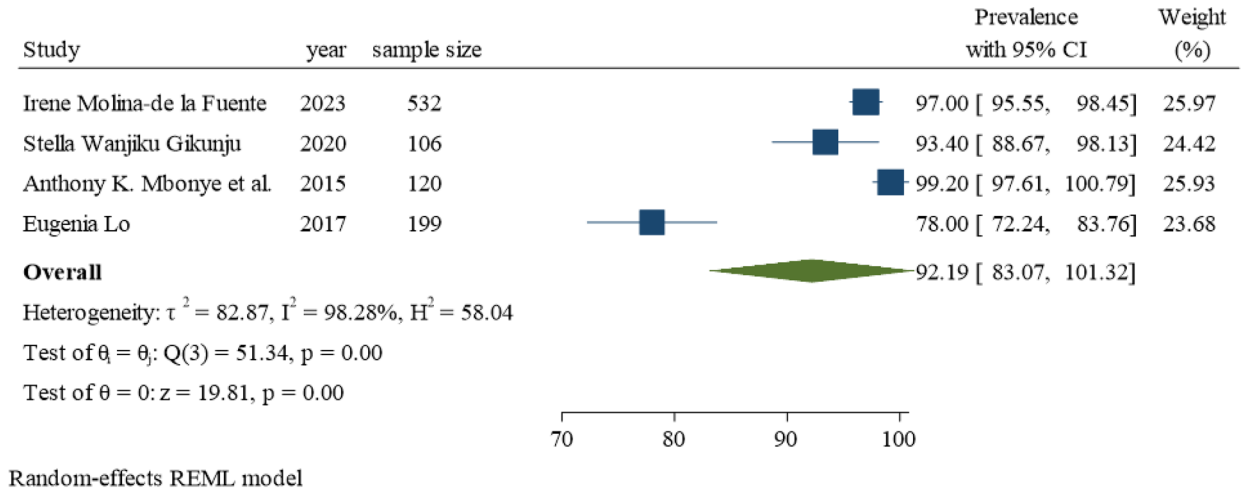


**Fig. 14** Forest plot showing the prevalence of dhfr 108 N mutation

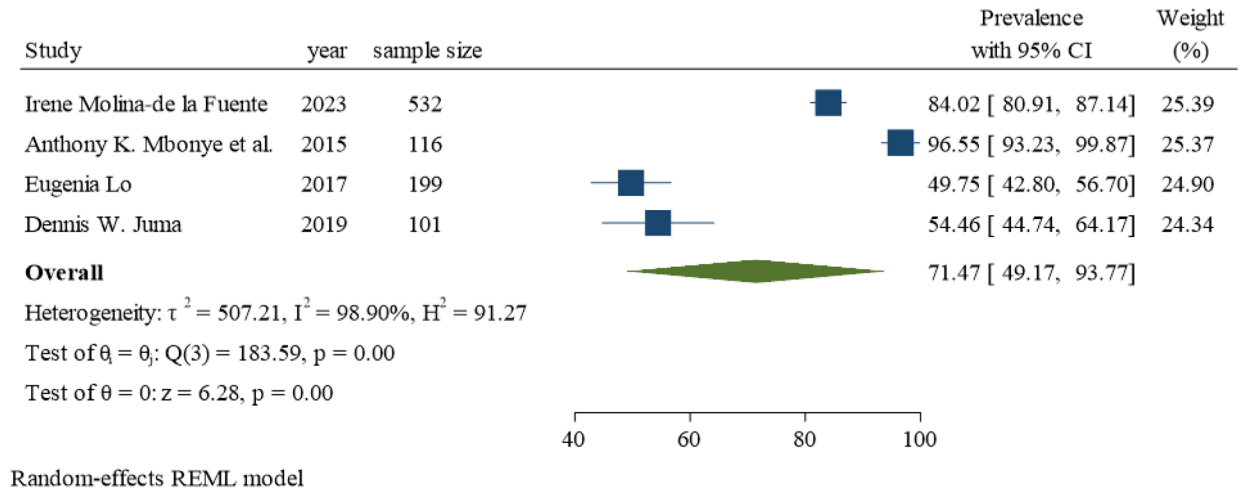


**Fig. 15** Forest plot showing the prevalence of dhfr 59R mutation

search engine. Both interventional and observational studies were retrieved for inclusion in the review. The following MeSH search terms were combined using the Boolean operators "OR" and "AND": "Prevalence", "Epidemiology", "Magnitude", "Biomarkers", "Molecular markers", "*plasmodium*", "*P. falciparum*", "*P. vivax*", "*P.ovale*", "*P. malariae*", "Drug resistance", "Antimalaria", "East Africa", "2014 to 2023".

## Eligibility criteria

### *Inclusion criteria*

The systematic review and meta-analysis covered the following types of studies: (a) papers published up to December 30,2023, on human participants of all ages; (b) original articles from studies that explored asymptomatic, uncomplicated, or severe malaria; (c) studies that included PCR genotyping of *P. falciparum* antimalarial drug resistance markers of SP; (d) research reporting the prevalence of molecular markers; (e) studies written in English; and (f) studies conducted in East African countries.

### *Exclusion criteria*

The following study types were excluded: (a) abstracts; (b) studies on in vitro, ex vivo, and in vivo antimalarial drug resistance without genotyping and reporting marker prevalence; (c) studies on genetic diversity and population structure of *P. falciparum* without drug resistance; (d) studies on diagnostic accuracy of methods for detecting

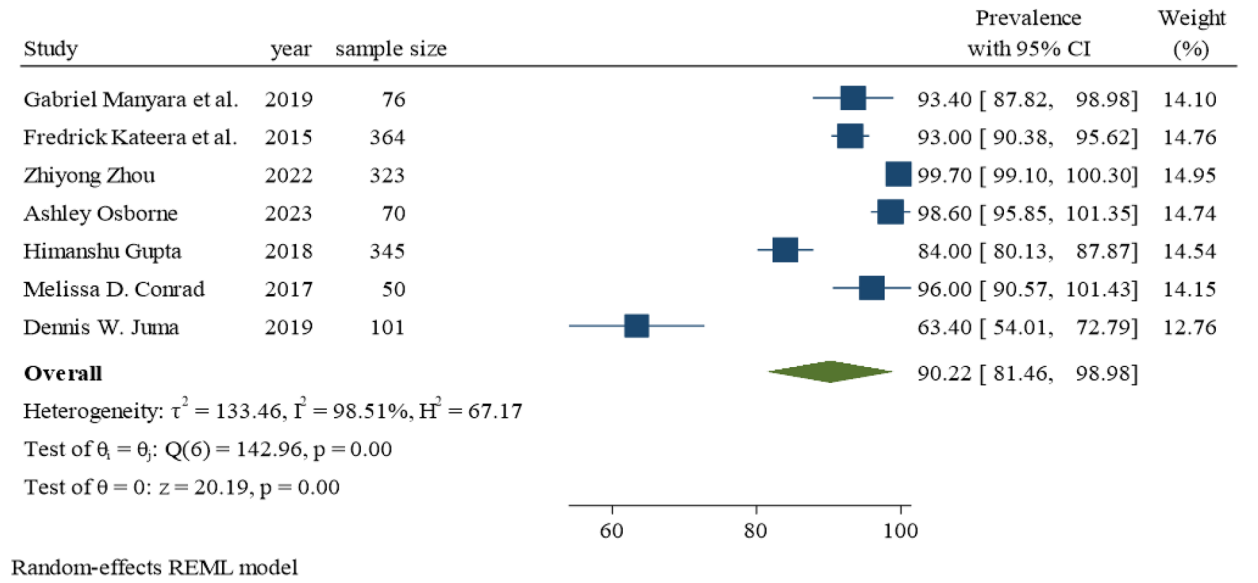


**Fig. 16** Forest plot showing the prevalence of dhps A437G mutation

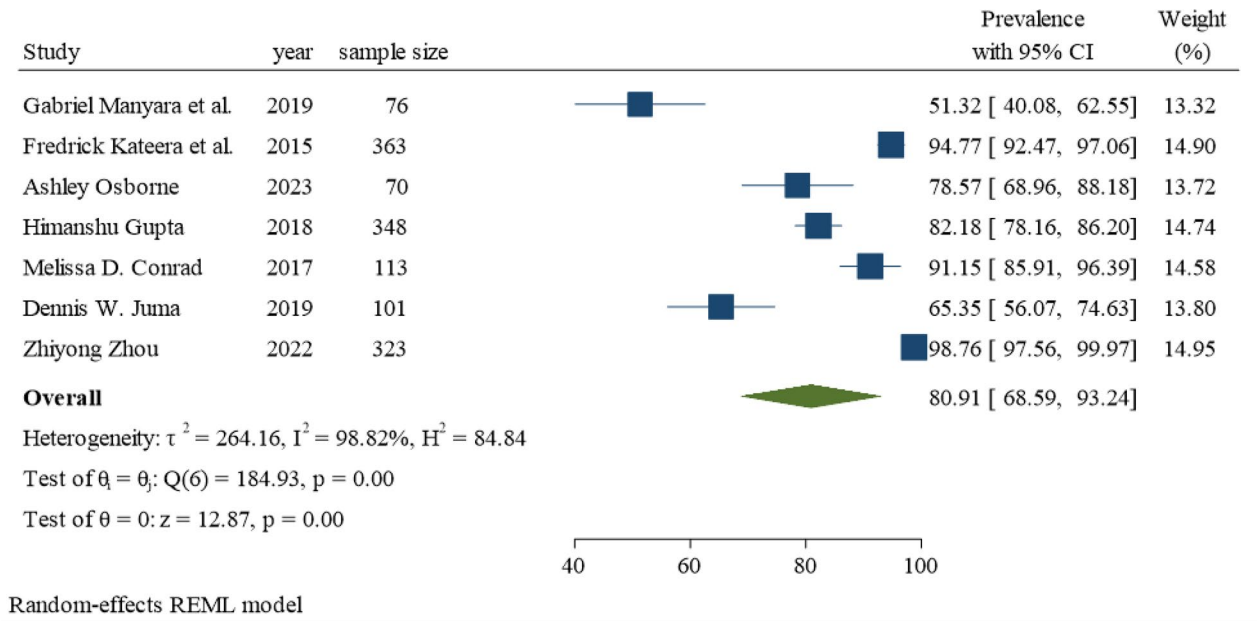


**Fig. 17** Forest plot showing the prevalence of dhps K540E mutation

*P. falciparum* without genotyping for antimalarial drug resistance of marker prevalence.

### *Review process*

Research articles found through electronic database searches were assessed for eligibility based on their titles, abstracts, and full text. The ineligible articles and duplicates were eventually removed. Before data extraction began, full-length articles from the selected studies were read to ensure that they met the inclusion criteria. Two independent reviewers (W.A. and Z.A.) inspected the titles and abstracts to identify potentially suitable studies, as well as data derived from full-length articles fulfilling the inclusion criteria.

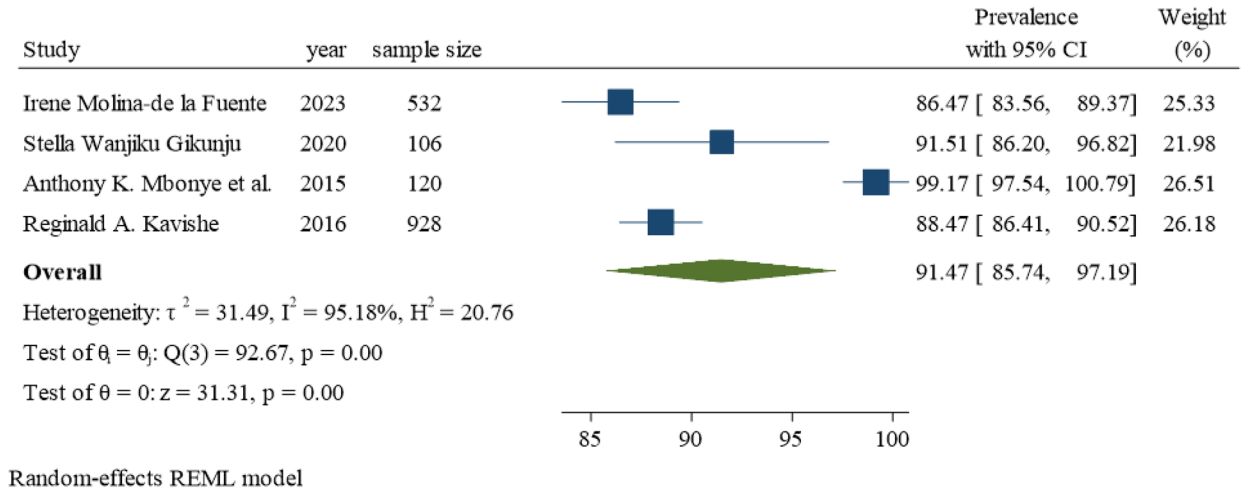


**Fig. 18** Forest plot showing the prevalence of dhps 540E mutation

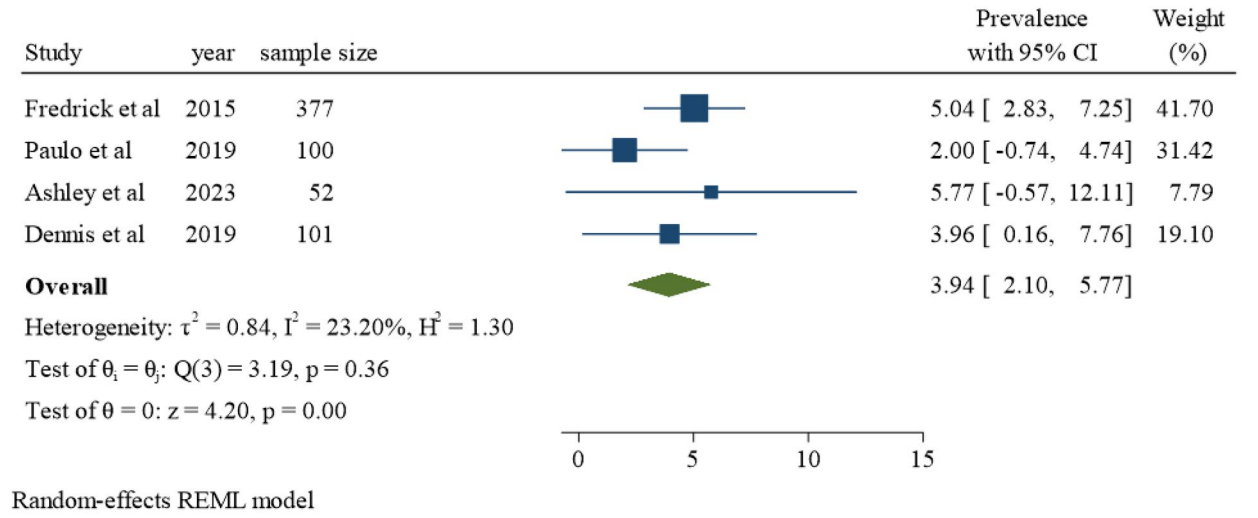


**Fig. 19** Forest plot showing the prevalence of dhfr I164L mutation

### *Outcome of interest*

The major outcome of interest was the prevalence of antimalarial drug resistance-conferring mutations associated with sulphadoxine-pyrimethamineine resistant *P. falciparum* in the original paper, expressed as a percentage and the number of cases (n)/total number of participants (N).

### *Quality assessment*

The quality of the articles was assessed using the Joanna Briggs Institute's (JBI) critical assessment checklist for simple prevalence [30]. Two independent investigators (G.K. and A.A.) assessed the quality of the full-text articles. Disputes were resolved through discussion to reach an agreement and accept or reject the articles for study. This systematic review and meta-analysis includes studies having a final quality score of at least 50%.

### *Data extraction procedure*

The relevant data extraction was done using Microsoft Excel, an established data extraction tool. This extracting tool contained information about the author(s)'names, study site, sample size, study design, sequence genotyping success rate, anti-malarial drug resistance gene (markers), total number of samples genotyped, number of samples genotyped with mutations, and prevalence of molecular markers. Five reviewers (W.A., A.S., M.N., M.A.R., and W.K.) assessed the extracted data for

**Table 11** Summary of subgroup analysis of *P. falciparum* molecular markers by year of publication and Country

| Variables | Characteristics | Molecular markers | Prevalence with 95% CI | $I^2$, p-value |
|---|---|---|---|---|
| Years of publication | 2014–2018 | dhfr N51I | 97.11 [93.48–100.75] | 75.96, < 0.001 |
| | | dhfr C59R | 90.57 [83.29–97.84] | 92.68, < 0.001 |
| | | dhfr S108 N | 96.45 [92.02–100.88] | 91.02, < 0.001 |
| | | dhpsA437G | 90.89 [83.92–97.85] | 89.86, < 0.001 |
| | | dhpsK540E | 89.45 [81.97–96.93] | 91.70, < 0.001 |
| | 2019–2023 | dhfr N51I | 82.03 [65.36,98.71] | 96.91, < 0.001 |
| | | dhfr C59R | 81.78 [67.19–96.37] | 96.72, < 0.001 |
| | | dhfr S108 N | 85.12 (72.04–98.21) | 96.76, < 0.001 |
| | | dhpsA437G | 89.24[72.98–105.50] | 99.13, < 0.001 |
| | | dhpsK540E | 73.98 [53.96–93.99] | 96.78, < 0.001 |
| Countries | Kenya | dhfr N51I | 85.88 [71.68,100.07] | 98.39, < 0.001 |
| | | dhfr C59R | 84.02 [68.27,99.77] | 98.53, < 0.001 |
| | | dhfr S108 N | 86.56 [72.73,100.38] | 98.54, < 0.001 |
| | | dhps A437G | 93.40 [87.82,98.98] | 98.72, < 0.001 |
| | | dhps K540E | 77.55 [60.77,94.34] | 97.68, < 0.001 |
| | Mozambique | dhps A437G | 84.00 [80.13,87.87] | 0, < 0.001 |
| | | dhfr N51I | 89.00 [82.87,95.13] | 0, < 0.001 |
| | | dhfr C59R | 86.00 [79.20,92.80] | 0, < 0.001 |
| | | dhfr S108 N | 90.00 [84.12,95.88] | 0, < 0.001 |
| | | dhps K540E | 82.12 [78.16,86.20] | 0, < 0.001 |
| | Rewanda | dhps A437G | 93.00 [90.38,95.62] | 0, < 0.001 |
| | | dhfr N51I | 99.70 [99.15,100.25] | 0, < 0.001 |
| | | dhfr C59R | 90.30 [87.33,93.27] | 0, < 0.001 |
| | | dhfr S108 N | 99.70 [99.15,100.25] | 0, < 0.001 |
| | | dhps K540E | 94.77 [92.47,97.06] | 0, < 0.001 |
| | Tanzania | dhfr C59R | 84 [79.58,88.42] | 0, < 0.001 |
| | | dhfr S108 N | 92 [88.73,95.27] | 0, < 0.001 |

correctness and consistency. The sixth reviewer (B.B.A.) was also consulted if needed.

### *Data analysis*

The relevant primary research was retrieved, imported into Microsoft Excel, and exported to STATA 17.0 software (StataCorp, Texas, USA) for final analysis. Forest plots were used to estimate the pooled effect size and effect of each study, along with their confidence interval (CI), and to generate a visual representation of the data. The inverse of variance ($I^2$) was used to evaluate the degree of heterogeneity among the included studies [31]. The inverse of variance ($I^2$) values of 25%, 50%, and 75% were thought to indicate low, medium, and high heterogeneity, respectively. The selected studies were assessed for potential publication bias using a funnel plot. Trim and fill meta-analyses were used to assess and adjust for the observed publication bias in the studies, as well as to estimate the number of potentially missing studies. We used a random effect model to analyze the pooled estimate because of the significant heterogeneity seen across studies. Studies with substantial heterogeneity were subjected to a subgroup analysis based on certain categories.

## Results

### Searching results

The electronic searches yielded a total of 9760 English-published articles and 2 unpublished articles on anti-*P. falciparum* drug resistance markers in East African countries. A total of 9544 studies were identified, after which 218 duplicates were removed. A total of 9544 studies were screened to remove studies by title, abstract, and full-text articles, with 16 studies retained after the screening and eligibility process. Finally, 16 studies were included for both qualitative and quantitative analyses (Fig. 1).

### Characteristics of included studies

This study encompasses participants of all ages and genders. A total of 16 studies were included in this systematic review and meta-analysis [28, 33–47]. Out of 16 studies included, 14 studies were obtained from

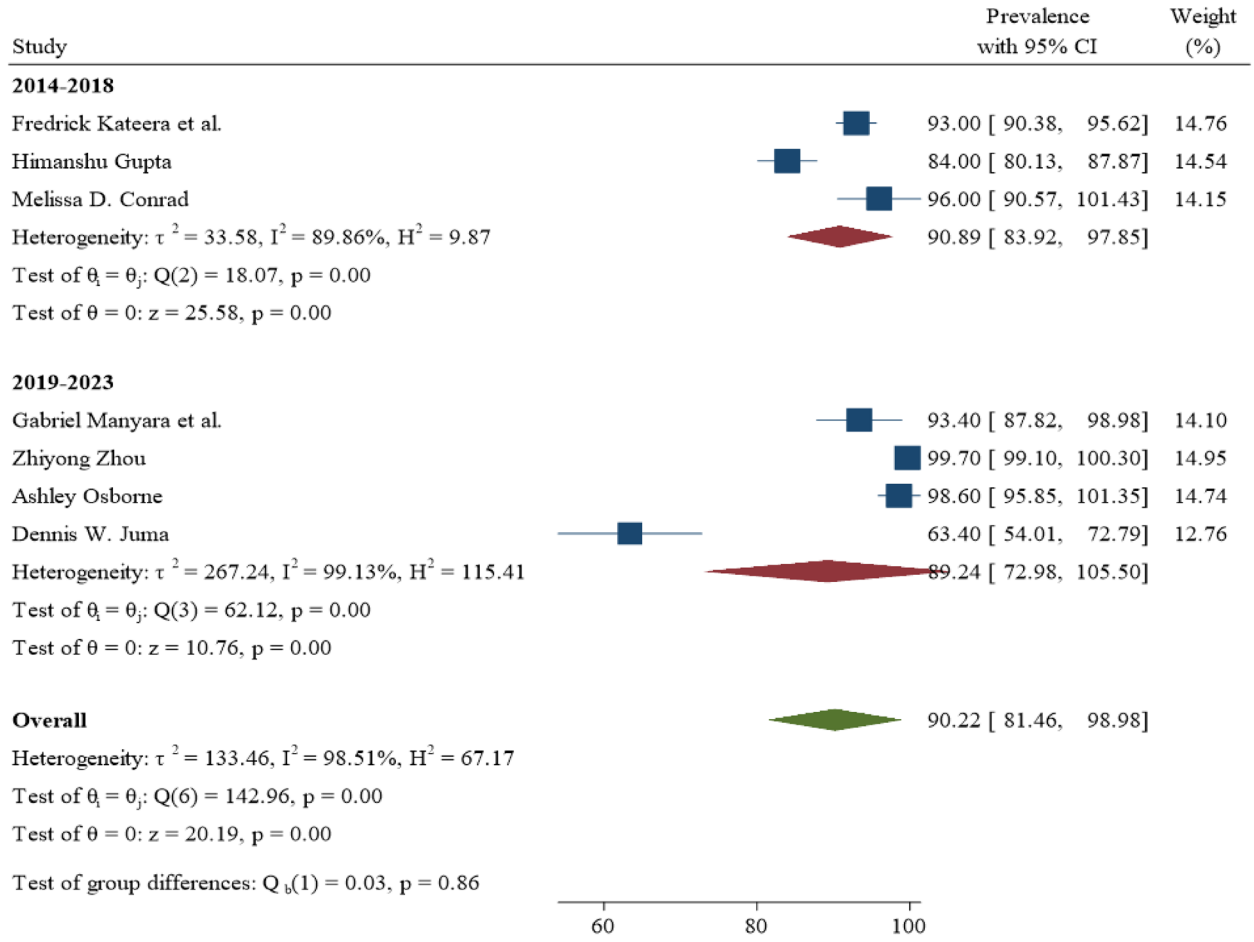


**Fig. 20** Subgroup analysis for pooled prevalence of dhps A437G mutations from 2014 to 2023

published articles [28, 33, 34, 37–41, 43–47] and 2 from unpublished data [35, 36]. Among the included studies most studies were from Kenya [28, 33, 37, 41, 45, 47]. Two primary regions determining resistance to SP antimalaria drugs were chosen, and quantitative synthesis was drawn, *Pfdhfr* (n = 14 studies) [28, 33, 35, 37–43, 45–48] and *Pfdhps* (n = 16 studies) [28, 33–35, 37–48]. Among the included studies 87.5% and 100% of them were studied Pfdhfr and Pfdhps, respectively (Table 1). Five studies reported the presence of double mutation with prevalence of (A437G-K540E) (84%), (59R-108 N) (97%), (437G/540E) (96%), (51I-108 N) (100%), (Gly −437 + Glu-540) (21%), (A437-K540E) (72%), and (437G-540E) (99%) [35, 36, 38, 40, 41]. Similarly, six studies reported the presence of triple mutation with a prevalence of (N51I-C59R-S108 N) (85%), [N51I-C59R-S108 N] (90%), (51I/59R/108 N) (88%), (51I-59R-108 N) (96%), (51I + 59R + 108 N) (4%), (Asn-108 + Ile-51 + Arg-59) (68%), [A437G- K540E-A581G] (79%), and (A437G-K540E-A581G) (84%) [35, 36, 40–42, 45]. Also, three studies reported the presence of quadruple mutation with prevalence of (N51I-C59R-S108 N-I164L) (4.5%), (51I + 59R + 108 N) + 437G] (10.4%), (59R + 108 N) + (437G + 540E) (10.4%), and (51I-59R- 108 N-437G) (97%) [36, 45]. Likewise, four studies reported the presence of quintuple mutant with prevalence of (N51I-C59R-S108 N-A437G-K540E) (63%), (51I-59R-108 N-437G-540E) (85%), (51I −59R −108N −437G-540E) (86%), and (51I −59R-108 N-437G −540E) (96%) [40, 41, 45, 47]. Moreover, Only one study reported the presence of sextuple mutant mutation with prevalence of (51I-59R-108 N) (437G-540E-581G) (8%) [40].

#### Heterogeneity and publication bias of included studies

The heterogeneity was assessed for all markers that are incorporated in different studies on *P. falciparum* markers that confer SP resistance. Except for dhfr I164L, there was significant heterogeneity across all markers, with $I^2$

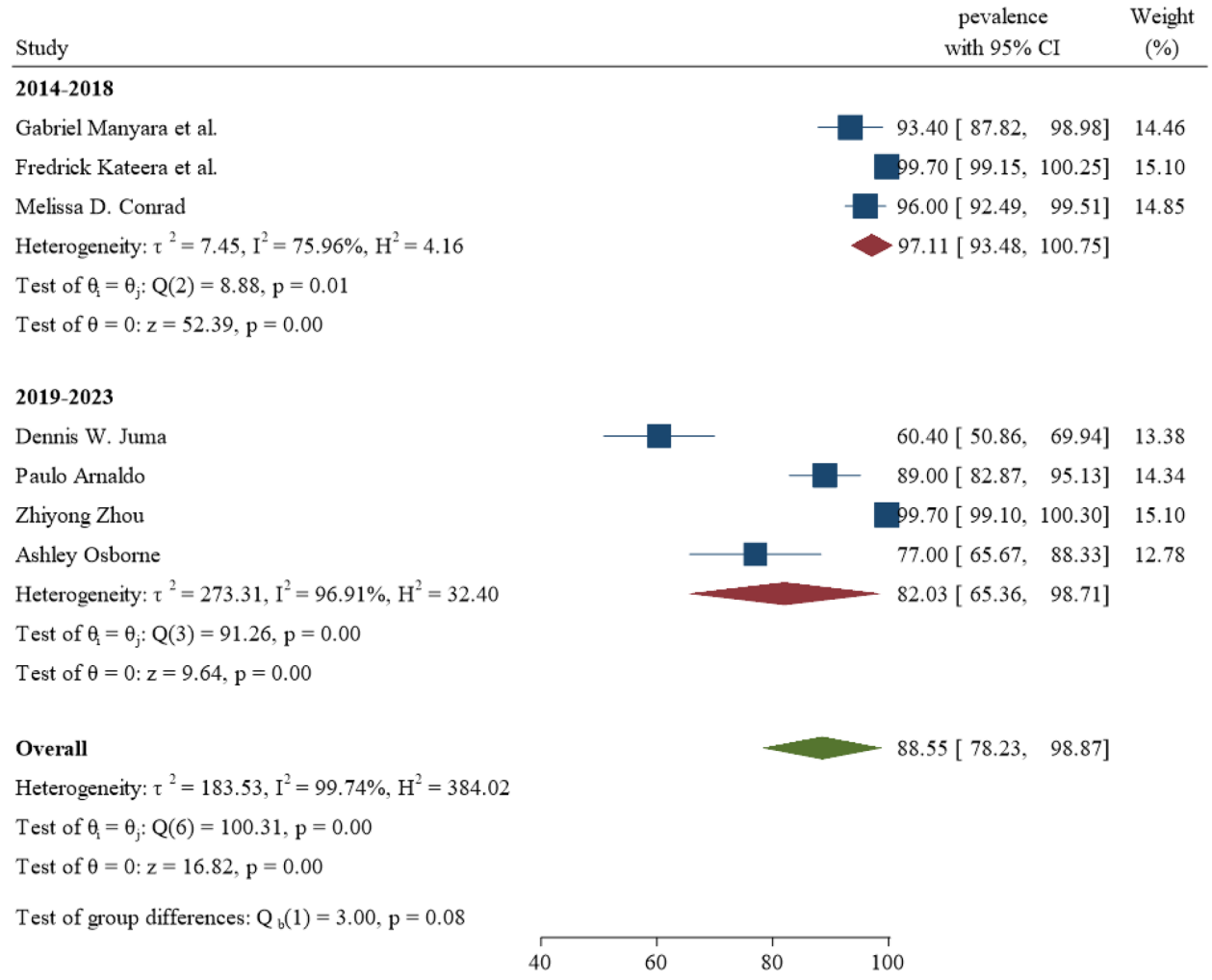


**Fig. 21** Subgroup analysis for pooled prevalence of dhps N51I mutations from 2014 to 2023

statistics showing higher than or equal to 95.18% at P value = 0.00. A funnel plot was used for assessing potential publication bias in the included studies. As a result, the funnel plot showed asymmetry, indicating that publication bias existed among studies. To reduce and correct for the observed publication bias in the studies, a trim and fill analysis was done to estimate the number of potentially missing studies. After adjusting for publication bias, the estimated pooled prevalence of dhfr N51I, dhfr C59R, dhfr S108 N, dhfr 108 N, dhfr 59R, dhps A437G, dhps K540E, dhps 540E, and dhfr I164L was 88.55 (95% CI 78.2–98.87), 81.1 (95% CI 71.98–90.2), 85.9 (95% CI 77.65–94.2), 89.7 (95% CI 81.1–98.3), 71.5(95% CI 49.2–93.77), 86.39 (95% CI 78.1–94.7), 80.9 (95% CI 68.6–3.2), 91.5 (95% CI 85.7- 97.2), and 3.8 (95% CI 2.1–5.51), respectively, based on trim and fill analysis Figs. 2, 3, 4, 5, 6, 7, 8, 9, 10 and Tables 2, 3, 4, 5, 6, 7, 8, 9, 10.

### Pooled prevalence of *P. falciparum* anti-malarial drug resistance determining mutations

The analyses of molecular markers revealed that the aggregated prevalence of dhfr N51I, dhfr C59R, dhfr S108 N, dhfr 108 N, dhfr 59R, and dhfr I164L, were 88.6% [95% CI 78.2–98.9], 85.3% [95% CI 76.1–94.6], 89.6% [95% CI = 81.1–98.2], 92.2% [95% CI 83.1–101.3%], 71.5% [95% CI 49.2–93.8], and 3.9% [95% CI 2.1- 5.77], respectively. Likewise, analyses of molecular markers revealed that the aggregated prevalence of dhps A437G, dhps K540E, and dhps 540E were 90.2% [95% CI 81.5–99], 80.9% [95% CI

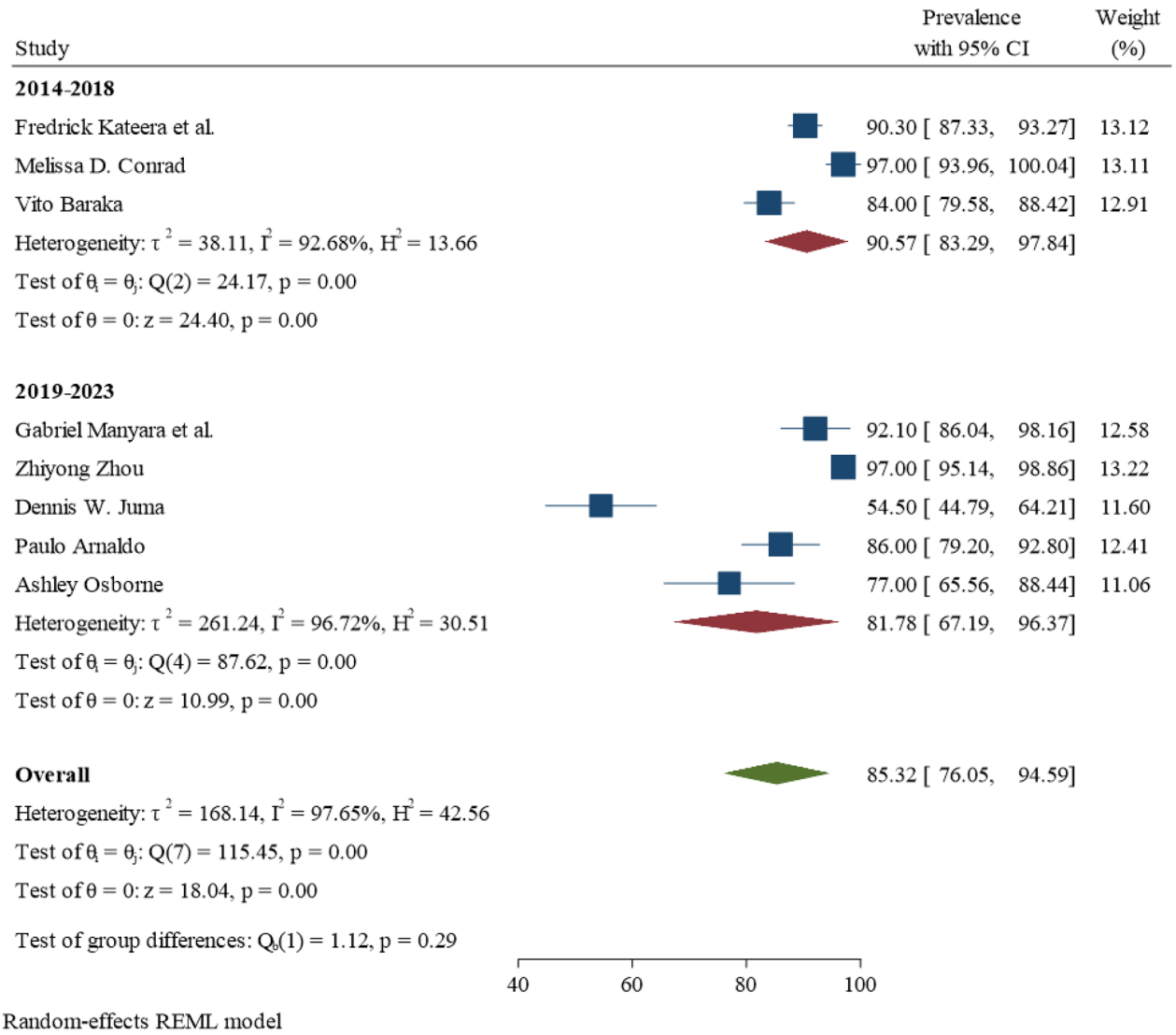


**Fig. 22** Subgroup analysis for pooled prevalence of dhfr C59R mutations from 2014 to 2023

68.6–93.2], and 91.5% [95% CI 85.7–97.2%], respectively (Figs. 11, 12, 13, 14, 15, 16, 17, 18, 19).

### Subgroup analysis of *P. falciparum* molecular marker by year of publication and country

There was high significant heterogeneity among the included studies. Inverse of variance ($I^2$) statistics showed greater than or equal to 95.18% heterogeneity among studies for all molecular marker like dhfr (N51I, C59R, S108 N, 108 N, and 59R) and dhps (A437G, K540E, & 540E). To identify the possible source of heterogeneity, subgroup analysis was performed for each molecular markers by year of publication and country. The subgroup analysis by year of publication analysis showing that the pooled prevalence of dhfr N51I, dhfr C59R, dhfr S108 N, dhps A437G, & dhps K540E, in 2014–2018 [97.11% (95% CI 93.48–100.75%), 90.57% (95% CI 83.29–97.84%), 96.45% (95% CI 92.02–100.88%), 90.89% (95% CI 83.92–97.85%), and 89.45% (95% CI 81.97–96.93%)], and 2019–2023 [82.03% (95% CI 65.36,98.71%), 81.78% (95% CI 67.19–96.37%), 85.12% (95% CI 72.04–98.21%), 89.24% (95% CI 72.98, 105.50%), and 73.98% (95% CI 53.96–93.99%)], respectively. A similar pattern was also observed on country based analysis showed that the pooled prevalence of dhfr N51I, dhfr C59R, dhfr S108 N, dhps A437G, & dhps K540E, in Kenya was 85.88% (95% CI 71.68,100.07%), 84.02% (95% CI 68.27,99.77%), 86.56% (95% CI = 72.73,100.38%), 90.7% (95% CI 78.02,103.39%), and 77.55% (95% CI 60.77–94.34%), respectively (Table 11) and (Figs. 20, 21, 22, 23, 24, 25, 26, 27, 28, 29).

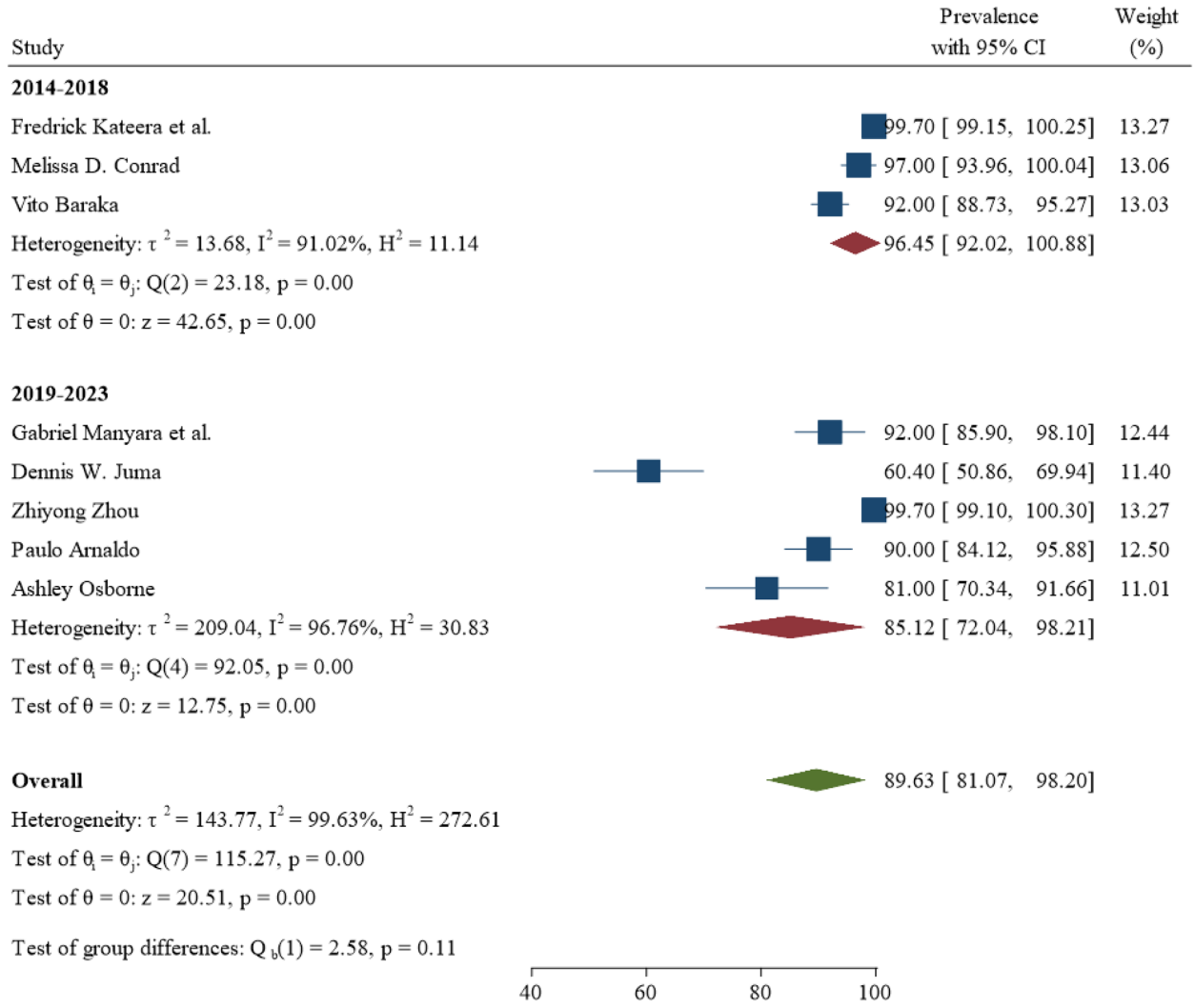


**Fig. 23** Subgroup analysis for pooled prevalence of dhfr S108 N mutations from 2014 to 2023

The meta-analysis showed no significant difference in all molecular markers prevalence like dhfr N51I, dhfr C59R, dhfr S108 N, dhps A437G, & dhps K540E among studies on year of publication. However, the meta-analysis showed significant difference in all molecular markers prevalence like dhfr N51I, dhfr S108 N, dhps A437G, & dhps K540E among studies on the country level except dhfr C59R.

## Discussion

This systematic review and meta-analysis showed the frequency of *P. falciparum* drug resistance markers of SP over a period of ten years in East Africa. In this systematic review and meta-analysis, the pooled prevalence of dhfr N51I was 88.6%. This finding was higher than that reported in Nepal [49] and Ghana [50]. This could be due to the widespread use of antifolate drugs, like SP, which can selectively influence the parasite population, causing drug-resistant mutations like dhfr N51I to occur and spread. Additionally, this may suggest that the region in problem is within a stratum with a high risk of malaria transmission and is an urban context with a high degree of variability and intensity in the use of anti-malarial medications and inadequate regulation. Also, this finding was inline with that reported in Senegal [51], Nigeria [52], Central African countries [53], and China [54]. This hypothesizes that the parasites are subjected to similar drug pressure in nations, or that the unrestricted movement of people for work and other purposes among

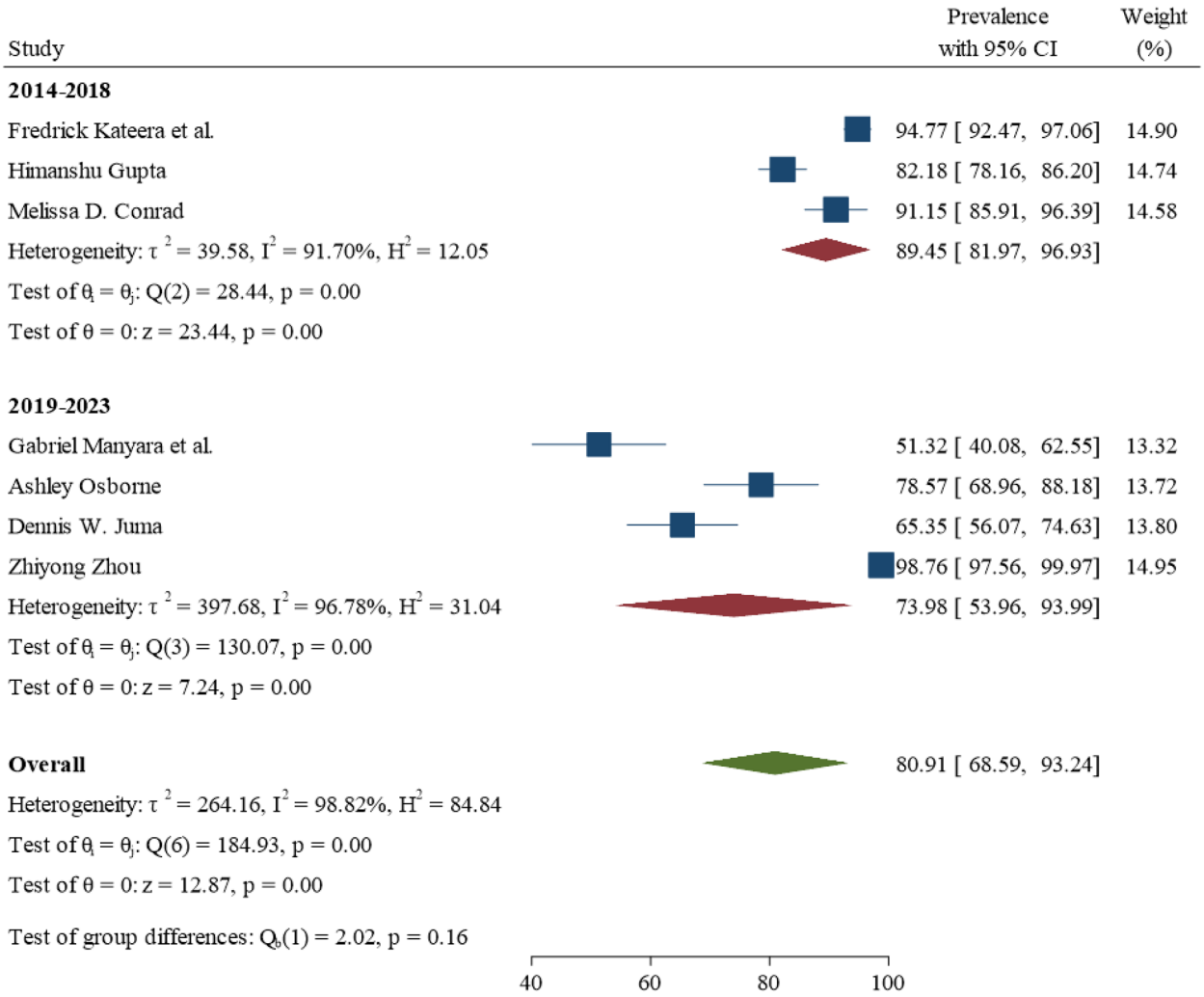


**Fig. 24** Subgroup analysis for pooled prevalence of dhps K540E mutations from 2014 to 2023

countries is responsible for the spread of parasites with similar drug resistance profiles.

Similarly, in this systematic review and meta-analysis, the pooled prevalence of dhfr C59R was 85.3%. This finding was inline with that reported in central African countries [53] and China [54]. But this finding was higher than that reported in India [55, 56], Senegal [51], and Ghana [50]. However, this finding was lower than that reported in Nepal [49], Mali [57], and Nigeria [52]. This might be due to the emergence and dissemination of drug resistance mutations like dhfr C59R which are caused by insufficient dosage, unfinished treatment regimens, or the use of substandard antimalarial medications. Furthermore, the high frequency of mutations could be attributed to the use of SP in groups like young children and pregnant women, who serve as reservoirs for infections with resistance allelles as a direct result of continuous use of SP in seasonal malaria chemotherapy and intermittent preventive treatment of malaria in pregnancy, initiatives that support the alleles' spread among the general population. Since SP is widely accessible at health centers and pharmacies in the study areas, its illegal use for self-medication may be a further major problem [58].

Likewise, in this review, the pooled prevalence of dhfr S108 N was 89.6%. This finding was lower than that reported in Central African Countries [53]. However, this finding was higher than that of reported in India [55, 56], Ghana [50], and Haiti [59]. This might be due to the parasite population in places with high genetic diversity is more likely to contain a range of drug-resistant mutations, such as dhfr S108 N. Furthermore, the large-scale deployment of intermittent preventive treatment for malaria prevention in pregnancy and seasonal

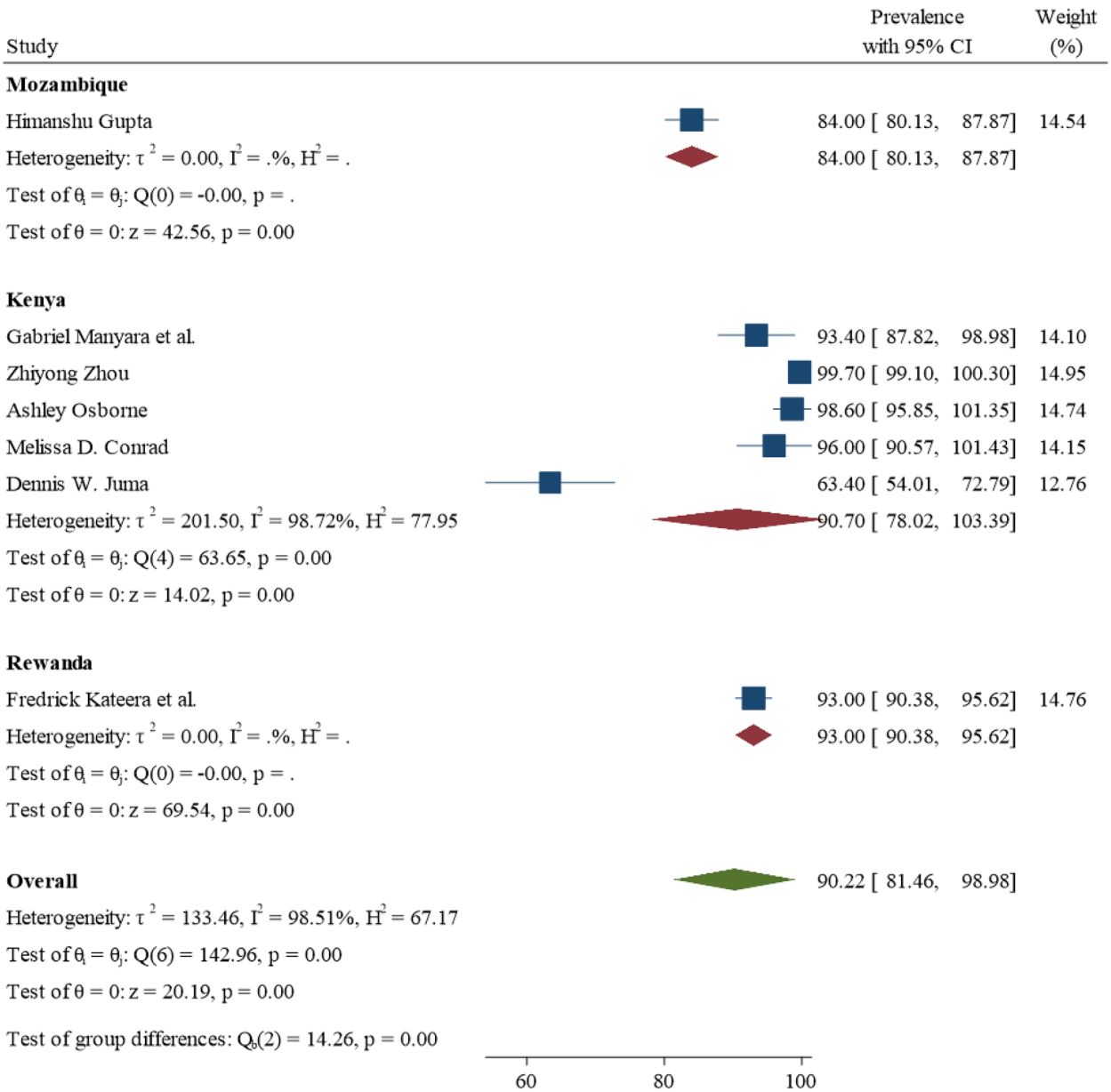


**Fig. 25** Subgroup analysis for pooled prevalence of dhps A437G mutations based on country

malaria chemotherapeutic treatments has undoubtedly contributed to the increase in drug pressure, which has promoted the propagation of parasite resistance to SP [60]. This finding was also consistent with that of reported in Nepal [49], Nigeria [52], and China [54].

Furthermore, in this review, the pooled prevalence of dhfr 108 N was 92.2%. This finding was similar to that reported in Cameroon [61], Sudan [62], and Ghana [50]. However, this finding was higher than that reported in Senegal [63]. This might be due to varying topographical variations and malaria transmission settings. Furthermore, this implies that SP selection is still going on in our study settings.

Moreover, in this review, the pooled prevalence of dhfr 59R was 71.5%. This finding was comparable with that of reported in Senegal [51, 63] and Ghana [50]. This finding was higher than that of reported in Sudan [62]. This might be due to drug resistance markers dispersed as a result of human and vector population movement within the same nation or across other nations. However, this

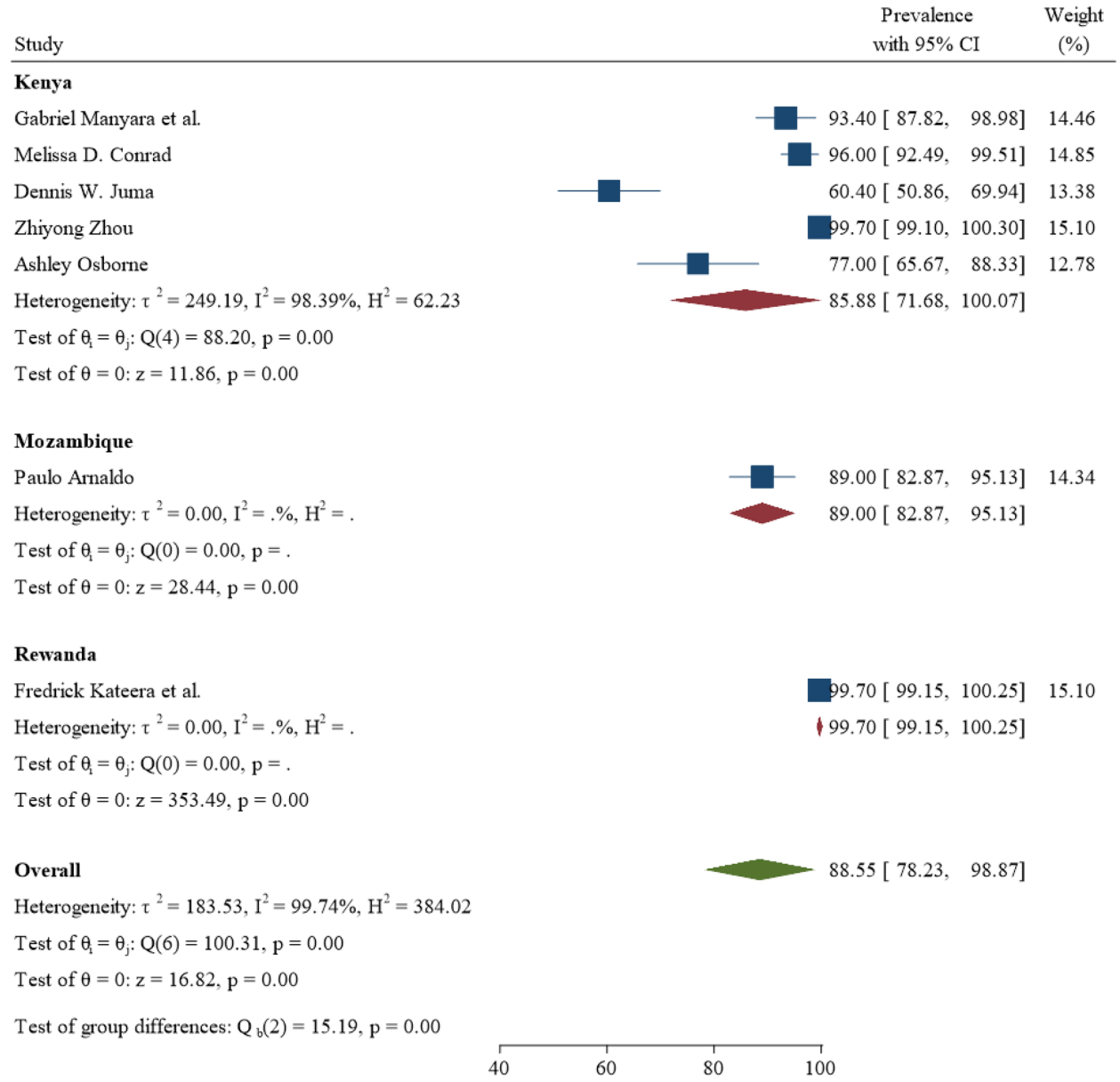


**Fig. 26** Subgroup analysis for pooled prevalence of dhfr N51I mutations based on country

finding was lower than that reported in Cameroon [61] and Mali [57]. This discrepancy between studies on the role of the dhfr 59R mutation in SP could be attributed to different study designs like in vitro studies, cross-sectional studies over time at the population level, or clinical trials testing drug levels in patients.

Additionally, in this review, the pooled prevalence of dhfr I164L was 3.9%. This finding was inline with that of reported in India [64]. However, this finding was lower than that reported in Malaysia [65], China [66], and Thailand [64]. Conversely, this finding was higher than that reported in Senegal [51] and Niger [67]. This might be due to the increased investment in road infrastructure throughout Sub-Saharan Africa, particularly in the Great Lakes region, the risk of the transmission of highly resistant mutations is larger than ever before [68].

Also, in this systematic review and meta-analysis, the pooled prevalence of dhps A637G was 90.2%, which is higher than that reported in India [55, 56], Nepal [49], Senegal [51], Mali [57], Cameroon [69], Nigeria [52], and

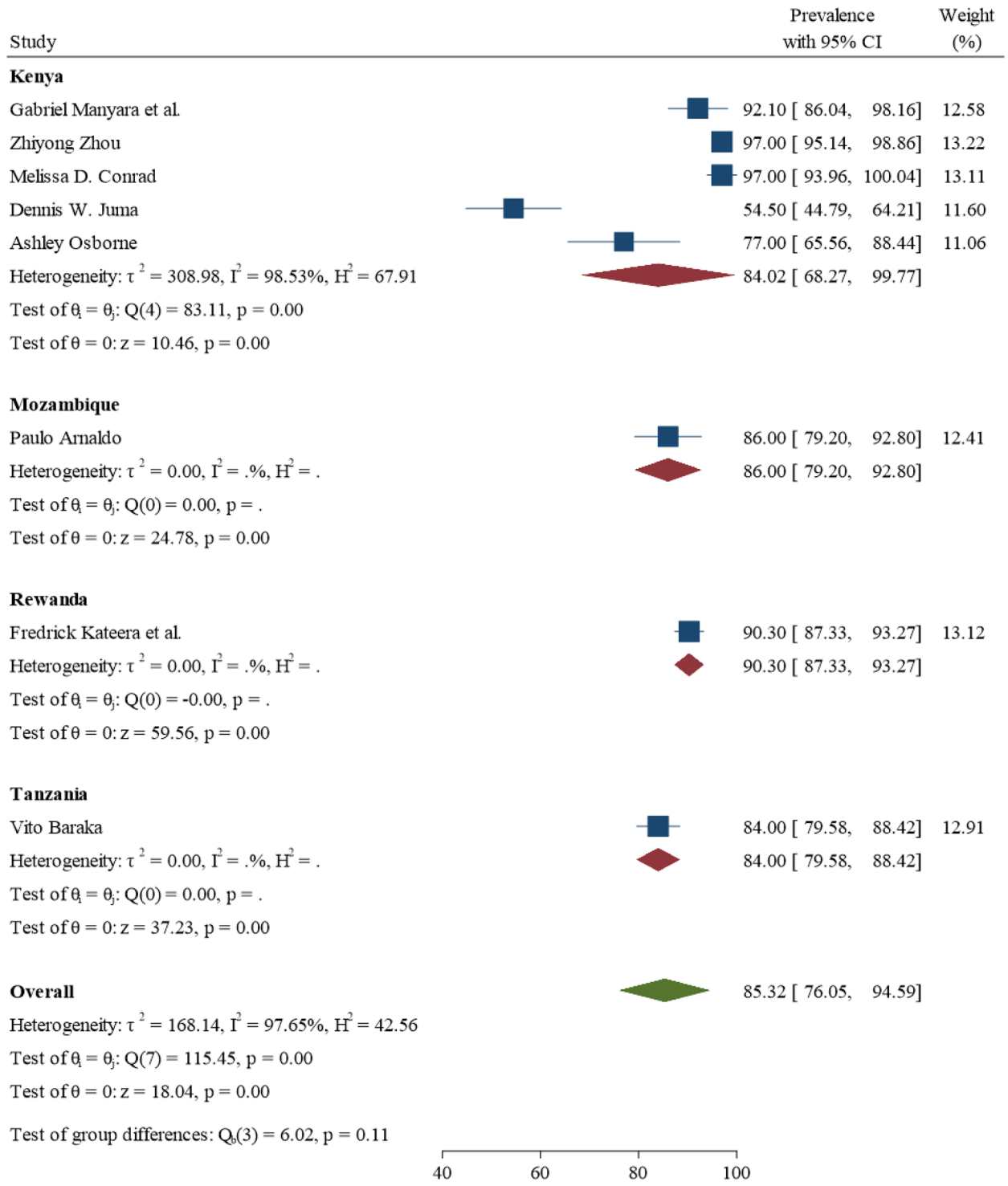


**Fig. 27** Subgroup analysis for pooled prevalence of dhfr C59R mutations based on country

Sierra Leone [70]. This could be a result of individuals moving about, which can help drug-resistant parasites spread from one area to another and contribute to the high occurrence of resistant strains like dhps A637G. It is well known that in Africa, the A437G mutation is highly linked to sulfadoxine resistance and a greater likelihood

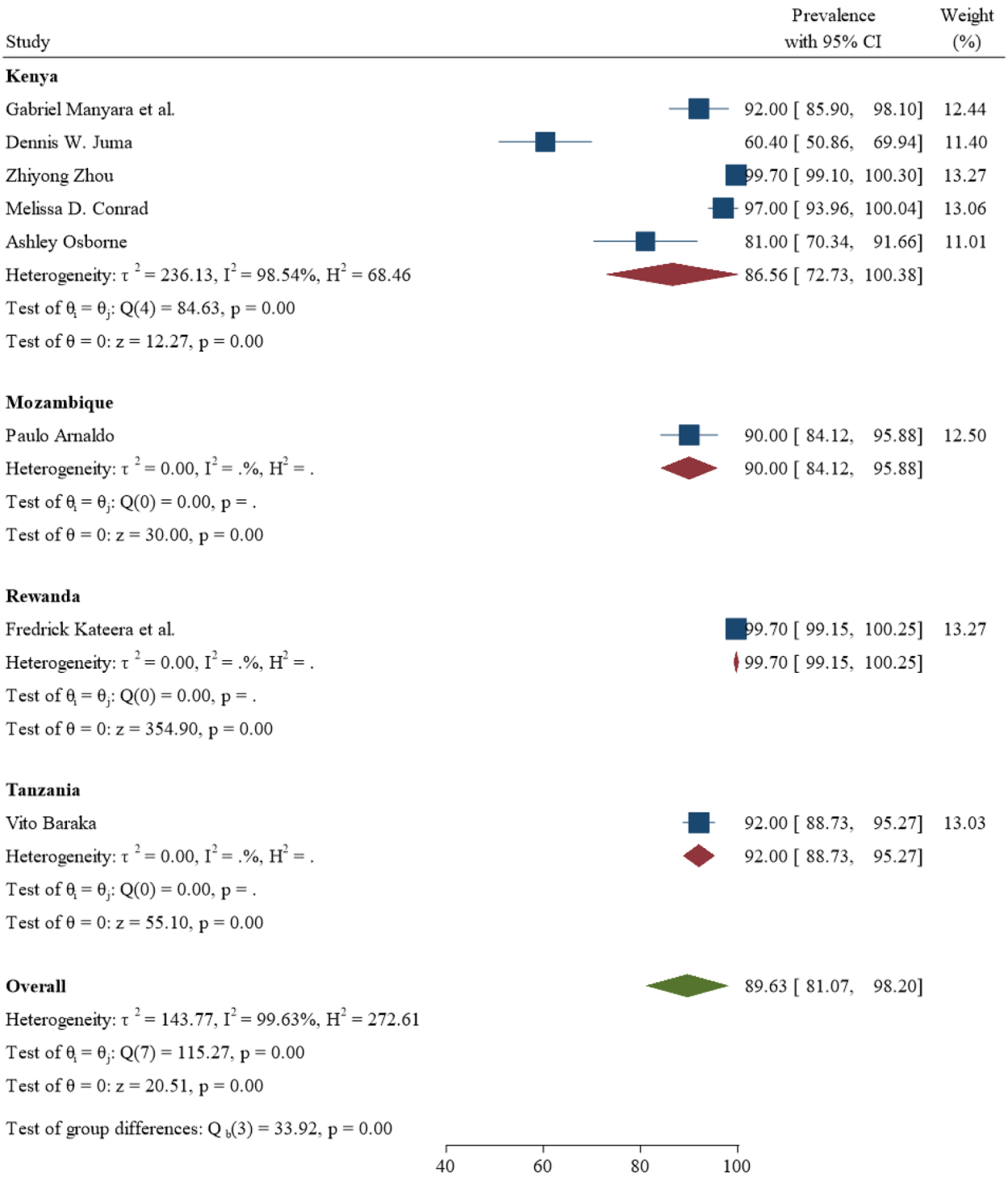


**Fig. 28** Subgroup analysis for pooled prevalence of dhfr S108 N mutations based on country

of failing SP treatment [52]. But this finding was inline with that of reported in central African countries [53], Congo [71], and China [54].

Similarly, in this systematic review and meta-analysis, the pooled prevalence of dhps K540E was 80.9%, which is higher than that reported in central African countries [53], Mali [57], Cameroon [69], Nigeria [52], and India

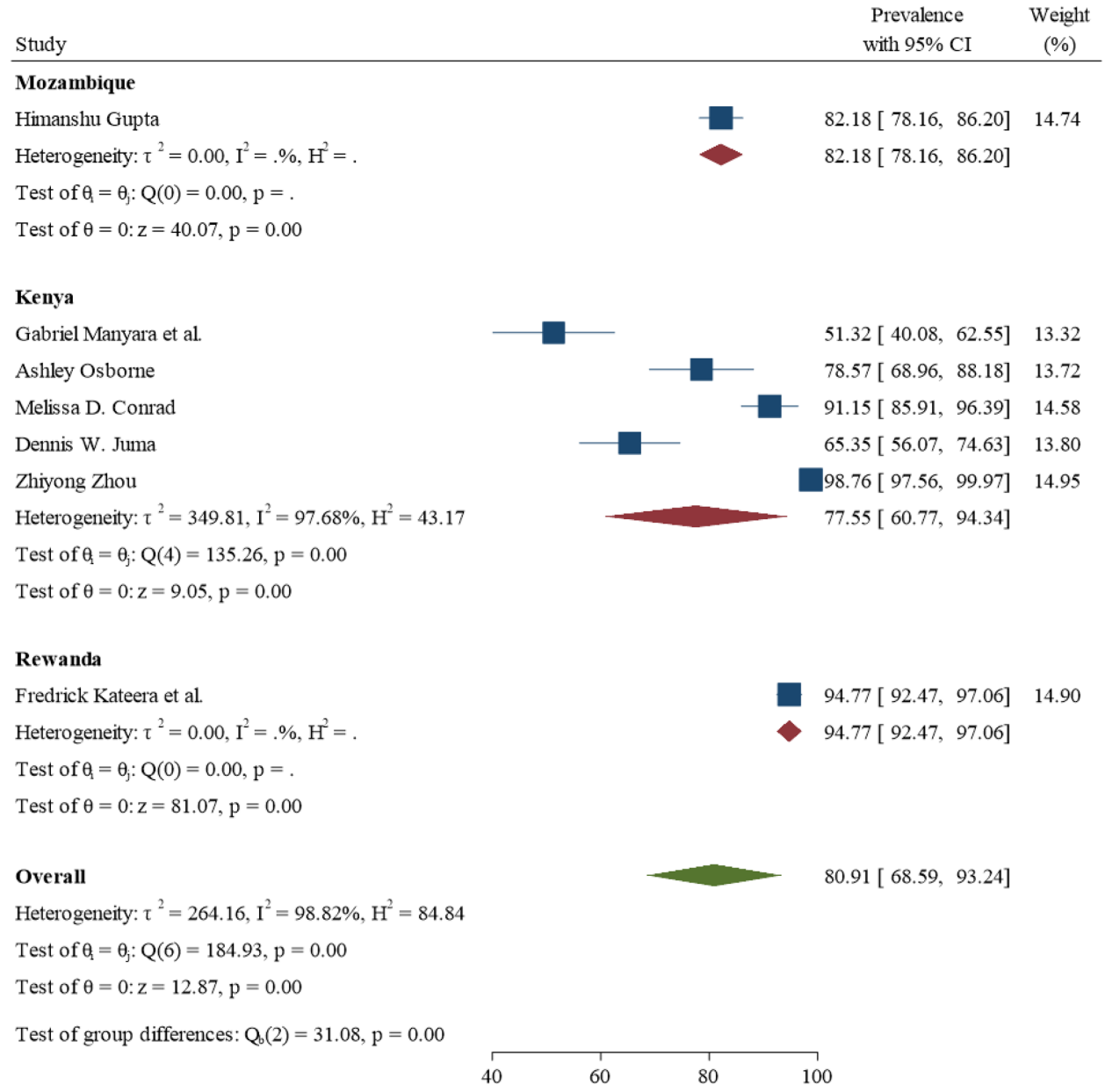


**Fig. 29** Subgroup analysis for pooled prevalence of dhps K540E mutations based on country

[55]. This might be because there are not many affordable or readily available alternatives to effective antimalarial drugs, which keeps people depending on antifolate drugs and encourages the selection of parasites that are resistant to treatments. This may also be due to malpractice in drug use, such as the use of the wrong dosage and insufficient information provided to patients about the prescribed treatment, which may lead to an increase in resistance and recurring infection rates. But this finding was inline with that of reported in in Nepal [49].

Likewise, in this review, the pooled prevalence of dhps 540E was 91.5%. This finding was higher than that reported in Cameroon [61] and Ghana [50]. However, this finding was lower than that reported in Sudan [62]. This could be the result of variations in sample sizes, patient status differences, and geographic differences. Furthermore, this suggests that either the parasites are subject to varying drug pressure among nations,

or the free movement of individuals between various nations for work and other reasons is the cause of the parasites'varied drug resistance profiles. Furthermore, the WHO still advises SP for the intermittent preventive treatment of pregnant women and their unborn children, although this recommendation has been discontinued in populations where 50% or more of the parasites have the dhps540E allele [3, 72]. The Pfdhps 540E has a high prevalence in East Africa [73, 74].

Moreover, in this review, double, triple, quadruple, quintuple, and sextuple mutants were reported in 44%, 50%, 25%, 25%, and 6% of studies, respectively. Correspondingly, these double, triple, quadruple, and quintuple mutants were reported in China [54], Myanmar [75], India [55, 76], and South America [77]. The main factor contributing to the rising frequency of double, triple, quintuple, quadruple, and sextuple mutations in *P. falciparum* is the pressure from drugs. Gene mutations cause resistance mechanisms in parasites when they are frequently exposed to antimalarial drugs. The drug's target site and capacity to enter the parasite or metabolic pathway could all be affected by these mutations. The presence of double, triple, quadruple, quintuple, and sextuple mutations in *P. falciparum* can lead to increased drug resistance. The frequency of these mutations varies geographically; double and triple mutations are more common in some regions, while quintuple and quadruple mutations are more common in others. Mutant genotype combinations are mostly associated with increasing resistance from double to quintuple mutations [73]. The spread of drug-resistant malaria is a serious public health concern because it can result in treatment failure and increased mortality [78, 79]. Furthermore, this systematic review and meta-analysis showed that a significant difference in the prevalence of molecular markers like dhfr N51I, dhfr S108 N, dhps A437G, & dhps K540E among studies on a country level. This shows that the distribution of these markers may vary spatially, with implications for understanding disease risk and creating targeted therapeutics.

### Strengths and limitations of the study

The major strength of the present review is that it has presented a picture of the prevalence and distribution of SP resistance markers of *P. falciparum* in East Africa with a total of 16 studies included. However, the data derived from this study did not include the pooled prevalence of dhfr and dhps gene muations. Since there was no stated prevalence of dhfr and dhps gene mutations in the included studies.

## Conclusions

The findings of this systematic review and meta-analysis regarding the markers of SP in East Africa revealed a significant prevalence of *P. falciparum* antimalarial drug resistance markers of SP. This indicates a substantial challenge in managing malaria infection caused by *P. falciparum*. The identified increase in the prevalence of antimalarial drug resistance markers of *P. falciparum* in SP leads to the widespread and quick emergence of drug resistance. This highlights the essential need for ongoing surveillance and research to create new antimalarial drugs and ways to overcome drug resistance. Also, different measures must be taken to prevent drug resistance with the remaining potent compounds as well as any new compounds that may be developed in the future. In addition, regular monitoring, identification, and limiting of drug-resistant *P. falciparum* strains through in vivo efficacy tests, in vitro tests, combination therapy, molecular techniques, and appropriate policies must continue to ensure the effectiveness of malaria treatment.

#### Abbreviations

| | |
|---|---|
| CI | Confidence interval |
| DHFR | Dihydrofolate reductase |
| DHPS | Dihydropteroate synthetase |
| PFDHFR | *Plasmodium Falciparum* Dihydrofolate Reductase |
| PFDHPS | *Plasmodium Falciparum* Dihydropteroate Synthetase |
| SP | Sulfadoxine-pyrimethamine |
| WHO | World Health Organization |

#### Author contributions

W.A led the systematic review and meta-analysis, overseeing the study's conceptualization, article selection, data extraction, statistical analysis, and manuscript preparation. W.A, T.M, A.A,T.E, and Z.D played a pivotal role in searching for relevant articles, conducting data extraction, performing statistical analysis, and contributing to manuscript drafting. B.BA,B.K,and A. AK were involved in statistical analysis consultation of the overall process of this systematic review and meta-analysis. G.K, M.N, A.J, Z.A, Y.G, E.G, M.G, A.S, M.AR, ST,S.G, W.K, M.A,and S.A involved in data mining, data extraction, in statistical analysis, manuscript writing, editing, and ensuring accuracy and completeness. Additionally, all authors actively engaged in critically reviewing the study's progress, data analysis, and manuscript preparation, involved in the approval of the final manuscript for submission, thereby affirming their endorsement of its content and findings.

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#### Availability of data and materials

No datasets were generated or analysed during the current study.

## Declarations

#### Ethics approval and consent to participate

Not applicable.

#### Consent for publication

Not applicable.

#### Competing interests

The authors declare no competing interests.

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## Publisher's Note