Degree project

UNIVERSITY OF SKÖVDE

Novel Therapy against Malaria Resistance using Meta-analysis

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Abstract

Malaria causes severe illness and death in some cases if not treated on time. The most vulnerable people are children and pregnant women in areas where it is rampant. The causative agent of the most severe malaria is *Plasmodium falciparum* this parasite is transmitted by the female anopheles mosquito when infected with *Plasmodium* parasites. Southeast Asia and sub Saharan Africa have the highest malaria death rate. Meta-analysis is one of the statistical tools used for estimating the mean and variance of underlying effects of a population under study from a collection of data from empirical studies addressing same research question. Meta-analysis has become an increasing valuable tool in research. This study describes the meta-analysis of novel antimalarial drugs. It involved selection of eligible articles based on certain inclusion criteria, calculating effect sizes, conducting the actual analysis using a popular software such as IBM SPSS and thus, estimating the effects of publication bias. This study identified three novel therapies used to treat Artemisinin Combination Therapy (ACT) resistant malaria. The resistance against ACT is developed in malaria due to mutation in K13 gene. It is evident that these different novel therapies in combination with ACT treatment can be used to treat resistant malaria and reduce the mortality rate.

Keywords: Malaria, Novel Drug, Resistance, ACT

Popular scientific summary

Malaria is a dangerous and occasionally fatal disease caused by a parasite that frequently infects a particular breed of mosquito that feeds on humans. Malaria symptoms are caused by the parasite, which infects people when they are bitten by a mosquito that transmits it. Malaria can cause a high fever, chills, flu-like symptoms, and anaemia, and it can be very dangerous for expecting moms. Malaria research is essential because in areas where it is prevalent, it poses a major risk to both public health and economic prosperity. Recent estimates place the annual death toll from malaria among children in Africa at one to three million. The novel therapeutic strategies in the present meta-analysis included newly discovered antimalarial agents that have a verified efficacy, potency and cost effective. The efficacies of the various novel therapies were evaluated and compared in the present meta-analytical study. The aim is to make available evidence-based research for Pharmaceutical industries. The main aim of the present study was to assess the novel therapeutic strategy against malaria. The study generated data from published articles. The comprehensive research was done by using different databases like PubMed, Science Direct, Google Scholar, MEDLINE and Research Gate to obtain the relevant research papers for this study. The key terms used for this research process were “Malaria” “novel therapy AND ACT resistant Malaria”. The inclusion criteria were studies that reported the effectiveness of the novel agents (drugs). However, studies that described other novel strategies aside drugs were excluded. After the selection of eligible articles, the following data were extracted using excel sheet: the author’s name, study year, novel therapy used, molecular marker of parasite targeted, study population, clinical trial phase of the study and efficacy of the novel drug for resistant malaria. The qualities of included articles were independently assessed by experts using the risk of a bias assessment tool for cross-sectional studies. The novel therapeutic strategies in the present meta-analysis evaluated newly discovered antimalarial agents that have verified efficacy, potency and cost effectiveness. The efficacies of the various novel therapies were evaluated and compared in the present meta-analytical study. It involves available evidence-based clinical findings. The outcome of the findings was analysed and reported in tables and figure comparisons from similar studies. To actualize the research aim, research questions were formulated by breaking them into different parts. Search terms were created by finding key articles that corresponded or had the same view as what was included in the search. Thus, relevant articles were selected and reviewed. The result revealed that three different novel therapies were used to treat Artemisinin Combination Therapy (ACT) resistant malaria. As discussed, the resistance against this ACT is developed in malaria due to mutation in K13 gene. The outcome of the study showed that the novel therapies in this review were spiroindolone KAE609, Se Compound therapy and RTS, S/S01 anti malaria vaccine. It concluded that these novel therapies have significant effectiveness to reduce paracitemia in the host body by targeting the K13 mutant genes, pfrTASe of parasite and targeting the different molecular biological pathways of the *p. falciparum* parasite. In conclusion, these different novel therapies in combination with ACT treatment can be used to treat resistant malaria and reduce the mortality rate.

Abbreviations

ACT - Artemisinin Combination Therapy

ART - Artemisinin

BSV - Blood-Stage Vaccines

DHA -Dihydroartemisin

GCP -Good Clinical Practice

GLP - Good Laboratory Practice

ITN - Insecticide Treated Nets

OR - Odd Ratio

PEV - Pre-erythrocytic vaccines

PICO - Population Intervention Comparison Outcome

WHO – World Health Organization

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Introduction

The only way human can be infected with malaria parasite is when bitten by a female anopheles mosquito. The illness can progress from mild to severe which can lead to death in some cases (Howick et al., 2019). Infected adults with malaria exhibit symptoms such as chills and fever which usually last for about 6 hours (incubation period) followed by sweating, headache, fatigue, stomach discomfort, and muscle pain. Symptoms in children include fever, cough, vomiting, and diarrhea (Meade & Watson, 2019). The most common causative agent of malaria infection in human is *Plasmodium falciparum.* This parasite has been reported to have developed resistance to several existing antimalarial drugs worldwide since more than five decades now (Simon-Oke et al., 2017). In the past, malaria parasite resistance was reported on drugs such as pyrimethamine, chloroquine, sulfadoxine, quinine and mefloquine (Noreen et al., 2021). The fight against malaria has made tremendous progress in the past 2 decades and this has reduced the death rate and morbidity by 50%. However, the death rate of *P.falciparum* accounts for about 500,000 yearly (WHO, 2016). This infection can be curbed or reduced depending on the availability and reliability of antimalarial drugs (Adedeji et al., 2020). Based on the urgent need to explore novel therapy in malaria treatment, the present meta-analysis will evaluate the novel antimalarial drugs for the treatment of malaria infection.

Meta-analysis is one of the statistical tools used for estimating the mean and variance of underlying effects of a population under study from a collection of data from empirical studies addressing same research question (Field & Gillett, 2010). Meta-analysis has become an increasing valuable tool in research. This study describes the meta-analysis of novel antimalarial drugs. It will involve selection of eligible articles based on certain inclusion criteria, calculating effect sizes, conducting the actual analysis using a popular computer packages such as IBM SPSS and thus, estimating the effects of publication bias.

Life cycle of Malaria Parasite

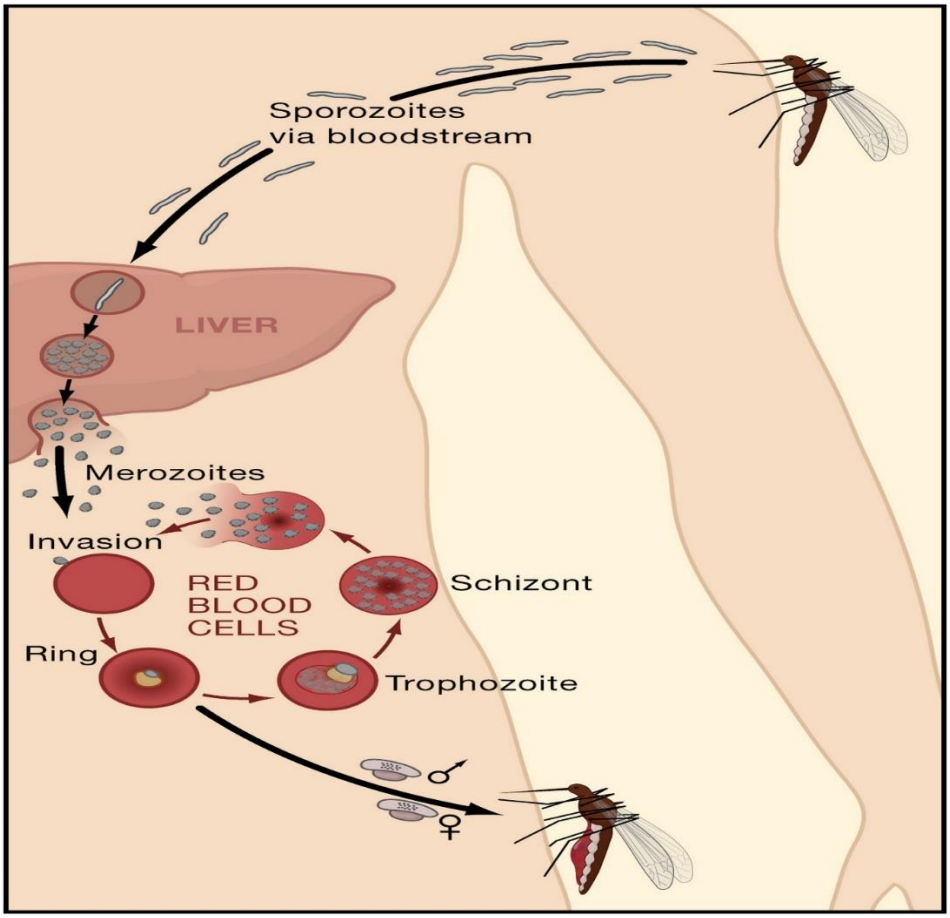

Figure 1. Life cycle of Malaria parasite adapted and modified from Zhang et al., 2015.

The host carrier of malaria parasites is the female *Anopheles mosquitoes*, which in turn infect humans. As female *Anopheles mosquitoes* take a blood meal for the purpose of nourishing their eggs, sporozoites are injected by the female *Anopheles mosquito* into the blood stream of the next human host. These sporozoites rapidly penetrate into the liver cells (exo-erythrocytic phase). The sporozoites develop to form schizonts from which thousands of merozoites develop from. However, in *Plasmodium ovale* and *vivax*, hypnozoites remain dormant in the hepatocytes phase. The parasite can remain dormant for a long period, months and years at the hypnozoites phase. *Plasmodium ovale* and vivax initiate the asexual cycle, thus causing clinical symptoms like fever, chills, etc in the absence of a new mosquito bite; hence, relapsing malaria is caused by *P. vivax* infection. Following the rupture of the liver cells, the merozoites are released and they swim into the bloodstream to rapidly invade the RBCs. At this stage, the parasites replicate asexually and lysing each red blood cell they infect, this leads to the clinical symptoms of malaria. A small percentage of merozoites develop into gametocytes, which are in turn taken up by the mosquito during another blood meal. The gametocytes cause the continuation of the cycle of transmission by the mosquito. The fusion of the male and female gametocytes takes place within the mosquito forming diploid zygotes, which develop into ookinetes. These ookinetes migrate to the midgut form the oocysts at the gut wall. This follows the formation of sporozoites when the oocytes divide meiotically. The sporozoites formed migrate to the salivary glands of the female *Anopheles mosquito* waiting for a bite for blood meal (Duffy et al., 2017).

Artemisinins and Artemisinin Combination Therapies (ACT)

Minor malaria can become severe and life threatening if left untreated. The old drugs used in treating this life threatening infection have become ineffective in some parts of the world due to resistance exhibited by the drugs (Sinclair et al., 2009). In the recent time, resistance to the most effective and the most recently approved antimalarial drug, artemisinin has been reported (Boudhar et al., 2016), by implication, the first-line therapy, artemisinin combination therapy, is becoming unsuccessful in some areas due to delayed parasite clearance and recrudescence. Artemisinin (ART) derivatives are artesunate, artemether, and dihydroartemisinin (DHA) (Heller & Roepe, 2019).

WHO currently approves 6 different ACTs (Artesunate-amodiaquine; artesunate-mefloquine; artesunate-pyronaridine; artesunate+sulfadoxine-pyrimethamine; artemether-lumefantrine; dihydroartemisinin-piperaquine). Out of theses 6 ACTs 2 injectable treatments (artesunate or artemether) are endorsed for the treatment of acute malaria and should be followed by an ACT when the patient can take oral therapy (WHO, 2021). Despite the fact that WHO recommended few ACT have been in circulation general due to reasons such as: Limiting elements are excessive cost, confined expertise and public consciousness at the idea of aggregate therapy (CT) and ACT in particular, confined expertise on protection of ACTs in pregnancy, operational difficulty which includes irrelevant drug use, loss of appropriate drug formulations, loss of post-advertising surveillance (PMS) systems, and the imbalance among call for and supply (Mutabingwa, 2005). Even with the limitations, possibilities are establishing up for powerful malaria control. The most common efficacious malaria controls that are easily accessible are the use of insecticides, insecticide treated nets (ITNs) and ACTs (Mutabingwa, 2005).

At some point during treatment if a parasite mutation generating resistance arises, the parasite ought to be killed with the aid of the partner drugs thereby decreasing resistance improvement to the artemisinin derivatives, and growing the beneficial lifetime of the individual drugs (Kunkel et al., 2021). In 2007, a clinical trial was demonstrated to show the efficacy of resistance to Art derivative, this trial showed the efficiency of artesunate monotherapy in treating patients infected with *P.falciparum* and this has helped in reducing the number of infected patients (Noedl et al., 2008). Artemisinin plays a very important role in treating malaria worldwide. Its derivative has been the most widely used in treating both serious and slight malaria in combination with other antimalarial drugs (Talman et al., 2019).

Artemisinin combination therapies (ACTs) are the most suitable first-line treatments for most *P. falciparum* infection in the whole world. Artemisinin derivatives kill a huge majority of parasites over numerous days with the aid of using one mechanism, and the associate drug removes residual parasites over numerous weeks with the aid of using an exceptional mechanism (Fairhurst, R. M., & Dondorp, A. M. 2016). Resistance due to Artemisinin Combination Therapy (ACT) has manifested as delayed parasite clearance and this resistance is mediated by Kelch (*K13*) gene (Rosenthal, 2020). The increase in the *P. falciparum* artemisinin-resistant strains has threatened public health and global efforts to eliminate malaria infections in line with the sustainable development goal (SDG).

Novel therapies such as the use of selenium (Se) based compound therapy for several diseases has recently been emphasized against these parasitic infections through targeting different key

pathways of parasite lifecycle. For scientists these parasitic diseases have become a challenge due to the resistance developed in this parasite against the previously first line treatment being used for malaria (Rashidi et al., 2022). Selenium based drugs or selenocompunds, in literatures show promising results for infectious and parasitic diseases. According to (Hariharan and Dharmaraj, 2020) selenium trace elements play significant function in human health owing to its anti-inflammatory, pro immune and anti-oxidant properties. Study conducted by (Bartolini et al., 2017) resulted that the selenium based compound reduces the cytotoxicity and perform its biological function by targeting the parasite selenoprotiens. Similarly, another study shows that selenium based compounds affects the redox homeostasis and cell signaling pathways of parasite cycle (Kurokawa and Berry, 2013). Several studies in literatures show that the selenium-based compound and selenoprotiens affect the host immune response. Furthermore the adequate amount of selenium based compounds through selenium therapy results in optimal level of IL-6 in malaria thus helps to manage the drug-resistantmalaria as an alternative to previously used first line malaria treatment by artemisinin and artemisinin combination therapies (Xia et al., 2021).

Another drug used as a novel therapy for artemisinin resistant malaria is spiroindolone as confirmed in different studies. In an exploratory phase 2 study done in Thailand, the spiroindolone antimalarial drug KAE609 indicated rapid effectiveness to treat *P. falciparum* and *vivax* malaria. The artemisinin have been established as the most rapidly acting antimalarial drugs till date; however, preliminary data suggest that KAE609 may cause even quicker pathogen elimination, especially in individuals with artemisinin-resistant *P. falciparum* infection (Zhang et al., 2015). According to H.Turner (2014), at low nano molar concentrations, spirotetrahydro—carbolines, or spiroindolones, are strong medications that destroy the blood stages of *P. falciparum* and *P. vivax* clinical isolates. In *P. falciparum*, spiroindolones limit protein synthesis quickly but this action is lost in parasites with point mutations in the gene expressing the P-type cation-transporter ATPase4 (PfATP4). In a rodent malaria model, the improved spiroindolone NITD609 displays single-dose effectiveness and pharmacokinetic features comparable with once-daily oral treatment. Spiroindolone/KAE609 is the first medicine which is clinically tested and that shows part of its antimalarial effect through biochemical breakdown of the infected RBC. It kills the parasite directly by blocking critical metabolic routes or through oxidative damage. This mechanism of dual destruction seems to be distinctive to the spiroindolone class of drugs, and it is prone to play a role in the remarkable and rapid clearance of parasite noticed, which is faster than that seen with artesunate, a drug that has no effect on the rheological characteristics of infected circulating RBCs with ring-stage parasitic infections (White et al., 2014). Given the impact of infected RBCs' increased deformability on their typical role and development in vivo, this trait underpins KAE609's potential as a key contributor to malaria prevention. It's a good medication combination in the struggle to mitigate and perhaps reduce the establishment of artesunate-resistant parasites since it increases elimination of RBCs strained with very early asexual ring-stage parasites that aren't susceptible to artemisinins. If further research shows that it also increases the removal of mature gametocytes, it will increase its efficacy as a transmission-blocking agent, which is a highly desired feature in future malaria-eradication treatments.

Another novel therapy for resistant malaria is vaccine development. Presently, there is no vaccine against malaria. It is generally acknowledged that newer methods, such as vaccinations, are

essential to maintain recent levels of controlling disease and progress towards malaria reduction and ultimately global extermination. Vaccine development efforts have focused on pre-erythrocytic (PE), blood stages, and sexual stages throughout the last 30 years. A truly viable Pre-erthrocytic vaccine will protect one from malaria by preventing the spread of infection to the bloodstream. Vaccines that target the asexual blood stages reproduction have been deemed critical for reducing mortality and morbidity. Vaccines that target sexual phases would disrupt the cycle of transmission but have no outcome on an infection that has already developed in the vaccine. The optimal antimalarial vaccine would just be found on specific targets that generate lifelong safety with few doses as possible in the early stages of life although this has not yet been accomplished. RTS,S/AS01, an anti-sporozoite vaccine, is the most advanced clinical candidate after successfully completing phase III clinical trials (Cowman et al., 2016).

A number of anti-malaria vaccines are now being developed in both clinical and pre-clinical trials, with a pivot on pregnant women and children. Pre-erythrocytic vaccines (PEV), blood-stage vaccines (BSV), transmission-blocking vaccines (MSTBV), and combination vaccinations target on various stages of life cycle of the malaria parasite. Despite a solid pipeline, these vaccinations are less likely to give long-time sterile protection against malaria, and will instead function as a tool to be used in conjunction with other interventions. Given the tremendous burden of disease, even a minor amount of protection from a vaccination would have a major effect, given the almost half-million deaths per annum (van den Berg et al., 2019).

RST,S/S01 is a pre-erythrocytic(PE) vaccine that was started to be developed since 1987 and has recently completed phase III testing. All parts of formal research standards, such as Good Clinical Practice (GCP) and Good Laboratory Practice (GLP) guidelines, were strictly followed in the phase III investigations, and every attempt was made to standardize the procedure. This huge multi-national experiment enrolled 15,459 newborns in 11 clinical trials centres across seven different nations including Burkina Faso, Gabon, Malawi, Mozambique, Ghana, Tanzania, Kenya. RTS,S has been demonstrated to be more effective in children of age 5–17 months who get three vaccine doses and booster dose at 20 months, resulting in a 36 percent reduction in severe malaria cases (Rts, 2015). When given with or without a booster dose, RTS,S/AS01 averted a significant number of cases of clinical malaria in young infants and children during a 3–4 year period. The delivery of a booster dosage improved efficacy in both age groups. As a result, when administered in conjunction with other effective control approaches, the vaccine has the potential to make a significant part to malaria control, particularly in places where transmission is strong (Rts, 2015).

Aim and Objectives

The main aim of the present study was to assess the novel therapeutic strategy against malaria.

The novel therapeutic strategies in the present meta-analysis will include newly discovered antimalarial agents that have a verified efficacy, potency and cost effective. The efficacies of the various novel therapies were evaluated and compared in the present meta-analytical study. The aim is to make available evidence-based research for Pharmaceutical industries. The outcome of the findings was combined and compared from similar studies.

To actualize the aim of the present study, the following specific objectives will be followed:

- To carry out literature review from published empirical studies that reported novel therapy in malaria treatment:
- To generate data from the articles showing the efficacy of the novel drugs.
- To carry out meta-analysis on the generated data.
- To make inference from the effect size of the statistical result.

Materials and methods

Meta-analysis is a statistical study that brings together the findings of several scientific investigations. When there are several studies done exploring same subject, and each research reports measurements that are predicted to have some degree of inaccuracy, meta-analyses can be done. For dichotomous outcomes, there are four extensively used meta-analysis approaches: three fixed-effect methods and one random-effect method. In this meta-analysis random effect model was used to conduct the analysis. The major difference between random effect approach and fixed effect approach is in the source of error. In fixed effect approach error occurs because of sampling from a population of studies whereas in random effect approach more error is created by sampling population from a super population (Schwarzer, Carpenter, & Rücker, 2015). The comprehensive research was done by using different databases like PubMed, Science Direct, Google Scholar, MEDLINE and ResearchGate to obtain the relevant research papers for this study. The key terms used for this research process were “Malaria” “novel therapy AND ACT resistant Malaria” Spiroindolone and Malaria”, “Selenium Compound and Malaria”, “Anti-Malarial Vaccine”, “RTS S/ASO1”. The research terms were used in English by using Bolean Operators “AND” and “OR”.

This research focused on systematic review and meta-analysis of already published data. The first thing done was a systematic review (full-text copies of all potentially relevant trials) of relevant on related topic. The review was done by following the few steps listed below:

- Formulated research questions by breaking it into different parts.
- Formulated or created search terms by finding key articles that corresponded or had the same view as what was included in the review.
- Searched in a structured way by using at least 2 databases such as Pubmed & Web of science for the search.
- Improved search strategy
- Selected and reviewed relevant articles.

The studies or papers reviewed had the same focus however the results were slightly different but the studies were related to an extent. All reviewed papers had the same effect size as well. Meta-analysis which is a statistical tool used to combine results from different scientific studies was used to determine the effect size for example odd ratios or relative risks with comparison of the results of the reported empirical studies. The articles were filtered using the criteria stated below.

Eligibility criteria

The inclusion criteria were articles that were written in English language and studies that reported the effectiveness of the novel agents (drugs). However, studies that described other novel strategies aside from drugs were excluded. Studies that evaluated symptomatic malaria infections and studies from other aspects of malaria treatment aside novel therapy were also excluded. The PICO (population, intervention, control, and outcomes) format is a widely known strategy for framing a research question (Aslam and Emmanuel, 2010). PICO approach was applied to gather the eligible studies. The population (P) will be patients diagnosed of malaria, intervention (I) will be the novel therapy, comparators (C) will be the positive and negative control groups that will be reported in the studies, outcome (O) will be the odd ratios based on the efficacy of the novel therapy; study design will be a meta-analysis.

Data sources and searches

Data collection was done based on the inclusion criteria set for this meta-analysis. The inclusion criteria set for this analysis was the inclusion of novel drugs therapy for resistant malaria against artesminine and its combination. Studies which were conducted on the seleneium or selenium based compound, spiroindolone and anti-malarial drug vaccine RTS,S/ASO1 for the novel anti-malarial therapy were used. Random effect model is suitable for sampling scattered data and for data which have variations. For sampling, random effect model was used to gather the studies conducted in this area resulting in the heterogeneity in the data collected. The databases used for extracting the previous studies in this area were the PubMed, Science direct and goggle scholar. A systematic literature search was done using PubMed, African Journal Online, Google scholar Scopus, Web of Science, and Embase to gather articles that discussed novel therapy in treatment of malaria infections. The search was done in English language.

Study selection and data extraction

All the studies were retrieved using databases search that are potentially relevant for inclusion. Duplicate articles were excluded, and then the titles and the abstracts of the eligible articles were screened. Also, the titles and abstracts which were not relevant to the study were excluded. The potentially relevant studies were scrutinized for full-text availability. The articles which looked useful and met inclusion criteria of the study were reviewed in depth by reading full paper. The reference lists of those articles were also carefully examined. After the selection of eligible articles, the following data were extracted using excel sheet: the author's name, study year, novel therapy used, molecular marker of parasite targeted, study population, clinical trial phase of the study and efficacy of the novel drug for resistant malaria.

Quality assessment

The qualities of included articles were independently assessed by experts using risk of a bias assessment tool for cross-sectional studies. The tool was used to evaluate both the internal and external validity. Some items such as data collection, proper case definition, validity and reliability of study instrument, was used to assess the internal quality, whereas sampling frame and random selection was used to evaluate the external validity.

Data analysis

All statistical analyses were carried out using Stata software (version 15). To synthesize the pooled odds ratios (ORs), the data was extracted in a tabular form. ORs and 95% Confidence Interval (CI) were calculated to evaluate the relationship between the efficacies of the novel drugs. The overall pooled ORs estimates were computed. Inconsistency index was used to assess the level of heterogeneity among the eligible studies. The study adopted a univariate meta-regression analysis based on publication years of eligible articles.

Table1. Characteristics of included studies

NT	CTP	Study	Effect Size	Outcome	CumPval
1	**2**	**Marina et.al., 2016**	0.1	3	0.96
1	**2**	**Nicholas et.al., 2014**	0.2	3	0.93
1	**2**	**Pawl et.al., 2013**	0.4	2	0.83
2	**1**	**Rashidi et al., 2022**	0.1	3	0.96
2	**2**	**Adebaye et.al., 2018**	0.2	3	0.93
2	**2**	**Zhang et.al., 2015**	0.2	2	0.91
3	**2**	**Robert et.al., 2021**	0.5	2	0.82
3	**3**	**Sanjev, 2014**	0.4	3	0.79
3	**3**	**Philip et.al., 2011**	0.8	3	0.71

Results

From the outcome of the review, about 110 related articles were accessed from various journal databases based on the keywords and about 90 articles were pooled from Google Scholar and PubMed. Among these articles, there were about 40 duplicates of which were removed about 44 articles did not meet the eligibility criteria. Thus, out of the 100 articles screened, 41 of the records were excluded because they do not meet the inclusion criteria of the research questions in the present review. The remaining 51 articles were sorted for retrieval, out of which, 33 articles were not retrieved. Out of the 26 articles that addressed the issue of antimalarial resistance therapy, only 9 reported novel drug designs in treating malaria resistance.

From the result in table 1, the effects size estimate of the sub-group revealed that spiroindolone/KAE609 has a value of 0.320±1.4552, while selenium based compound has the least effect size of about 0.169±1.4552. The vaccine candidate (RTS,S/SO1 Vaccine) has the highest effect size of about 0.537±1.4552. From the outcome of the findings in the present review, the overall effect size projected was 0.342. The effect size of the novel therapy in malaria resistance treatment in the present study varied and can be classified in line with the guideline postulated by Hattie, John; & Hamilton, (2020). It was opined that an effect size of 0.2 may be inferred to have a small effect, effect size of 0.4 have a medium effect and effect size of 0.6 is said to have a large effect on the research findings. According to Hattie et al., (2020), an effect size of 0.4 is described as the hinge point, at which the effects of the outcome can be said to have greater than or equal to the mean influence on the targeted population. Based on this classification, spiroindolone/KAE609 with an estimated effect size of 0.3 can be inferred to have an effect below average in the Hittatie guideline, and the effect size is also slightly less than the overall effect size in the review. Selenium-based compound on the other hand fell under the category of small effect

size with an effect size of about 0.16. This value is also less than the overall effect size in the review. However, the RTS, S/SO1 Vaccine has an effect size classified as large effect based on the Hittatie guideline. The effect size is greater than the overall size effect in the study. According to Rothwell et al., (2018), effect size of 0.30 is referred to as the median standardized effect size with an interquartile range of 0.20 to 0.38, while effect size of 1.18 is referred to as the maximum effect. It can also be seen from the outcome of the study that spiroindolone has a p-value of 0.826, selenium compound has p = 0.908 level of significant and RTS SO1 has a significant level of p = 0.712. By implication, there is no statistically significant difference.

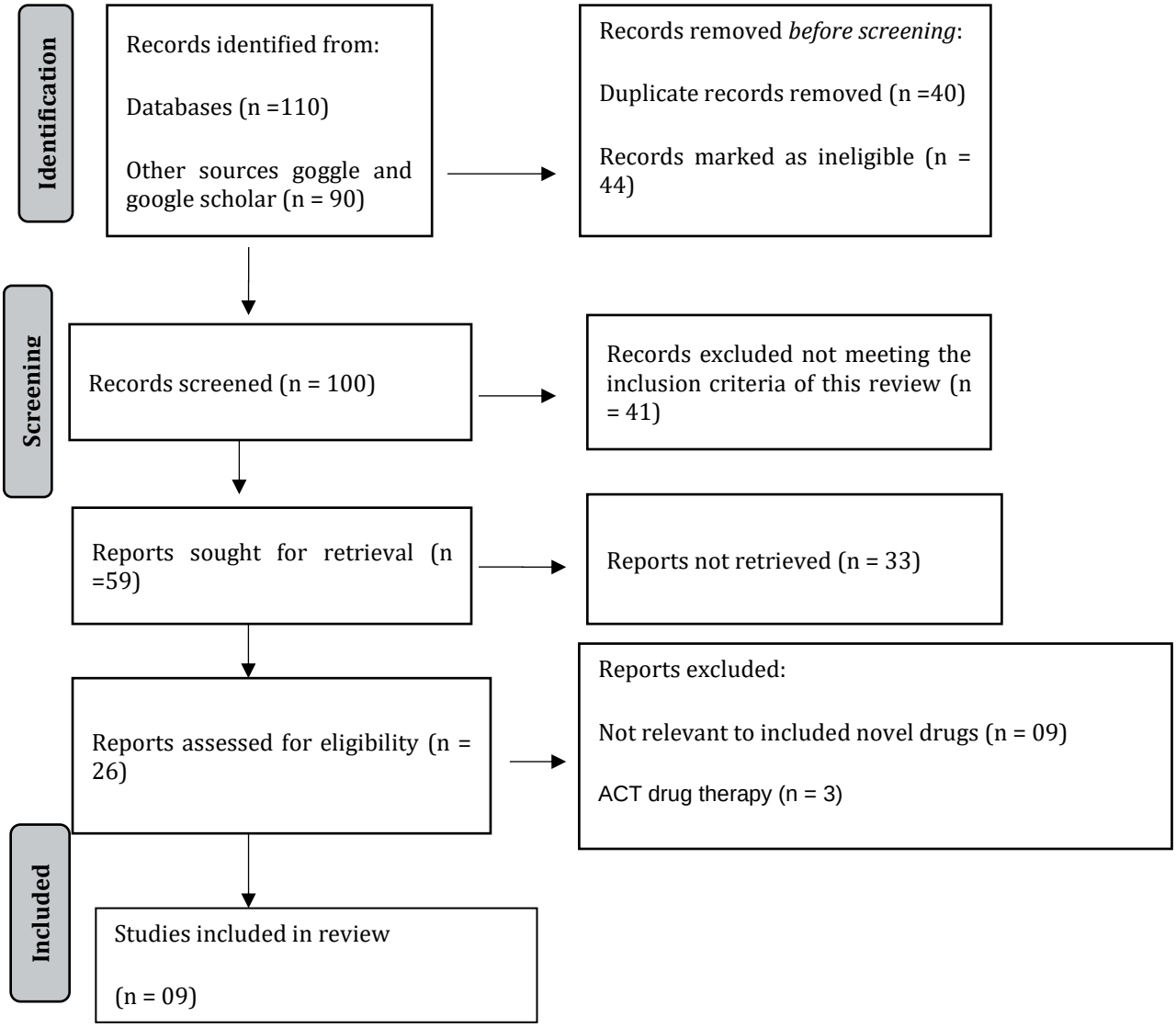

Figure 2. PRISMA (preferred reporting items for systemic reviews and meta-analysis) flow chart for screened studies.

From figure 2 above, it can be shown that 200 articles were assessed out of which 40 duplicates and 44 were marked as ineligible. The records screened were 100, out of which 59 were retrieved. The articles assessed foe eligibility were 26, 9 were not relevant to be included as novel drugs, 3 were ACT therapy and 5 were not clear. A total of 9 studies were used.

In this study three novel therapies were reported to have the efficacy to treat the Artemisinin Combination Therapy (ACT) resistant malaria. As discussed, the resistant against this ACT is developed in malaria due to mutation in K13 gene. The novel therapies reported by the eligible studies are Spiroindolone, Selenium based compounds and RTS, S/SO1 vaccine. Table 2 illustrates the combined effect size (odd ratio OR) of the spiroindolone studies is 0.320, for selenium based studies OR is 0.169 and for RTS,S/SO1 it is 0.537. Std. Error of all three novel therapies used to treat the resistant malaria are the same i.e 1.4552 as it was applied on the variable of clinical trial phase of that study and included studies for each therapy have almost similar number of clinical trial phase and thus showing same standard error of that variable.

Table 2. Effect Size Estimates for subgroup Analysis

	Effect Size	**Std. Error**	**Z**	**Sig. (2-tailed)**	**95% Confidence Interval**	
Spiroindolone/KAE609	0.320	1.4552	0.220	0.826	-2.532	3.172
Selenium based compound	0.169	1.4552	0.116	0.908	-2.683	3.021
RTS,S/SO1 Vaccine	0.537	1.4552	0.369	0.712	-2.315	3.389
Overall	0.342	0.8402	0.407	0.684	-1.305	1.989

Forest plot of the different studies with their effect size, and outcomes efficacy. The effect size of the revised studies is also shown in figure 3 below on forest plot which is < 1.

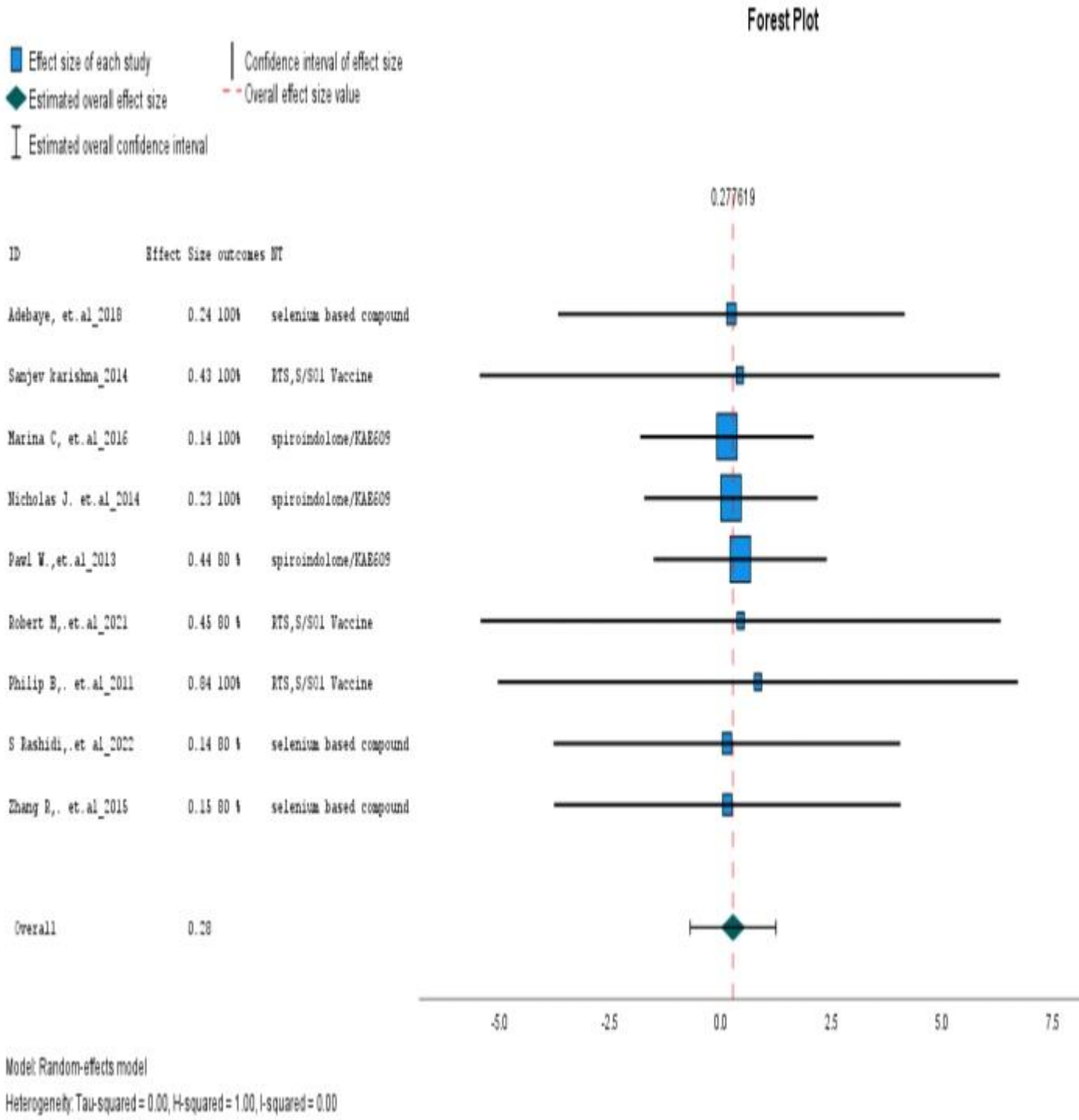

Figure 3. The forest plot summarizing the results from the meta-analysis. Each study in the meta-analysis is represented by the point estimate with a horizontal line passing through the box. The boxes are of different sizes representing the article weight. From the result, Spiroindalone/KAE605 was reported by three articles with effect sizes of 0.14, 0.23, and 0.44. Thus, the studies provide more information on spiroindalone than other agents. The effect size of selenium was 0.24, 0.42. The overall confidence interval is 0.28.

The forest plot in figure 3 provides information about the heterogeneity of the eligible studies. Since several primary studies are brought together to provide one estimate, there are variability

among the studies with respect to the participants, treatments and outcomes. In the present systematic review, the articles could be the same outcome but have varying results that are inconsistent with each other. Some of the report may have more effect than another. In another scenario, the report varied in measurement of the odd ratio outcomes. In the case of this systematic review geared towards evaluating the efficacy of a novel therapy in malaria resistance, the clinical trials that evaluated the effects of the novel therapy ranges from odds ratios of 0.24 to 0.45 favouring the outcome of the novel therapy. Theoretically, the odd ratios of the outcomes could be added all together to arrive at a single sum, but the average value would not apply to all the varying interventions from the novel therapy. Thus, the clinical heterogeneity occurred in the present review.

The studies used in the meta-analysis are also shown in the funnel plot below.

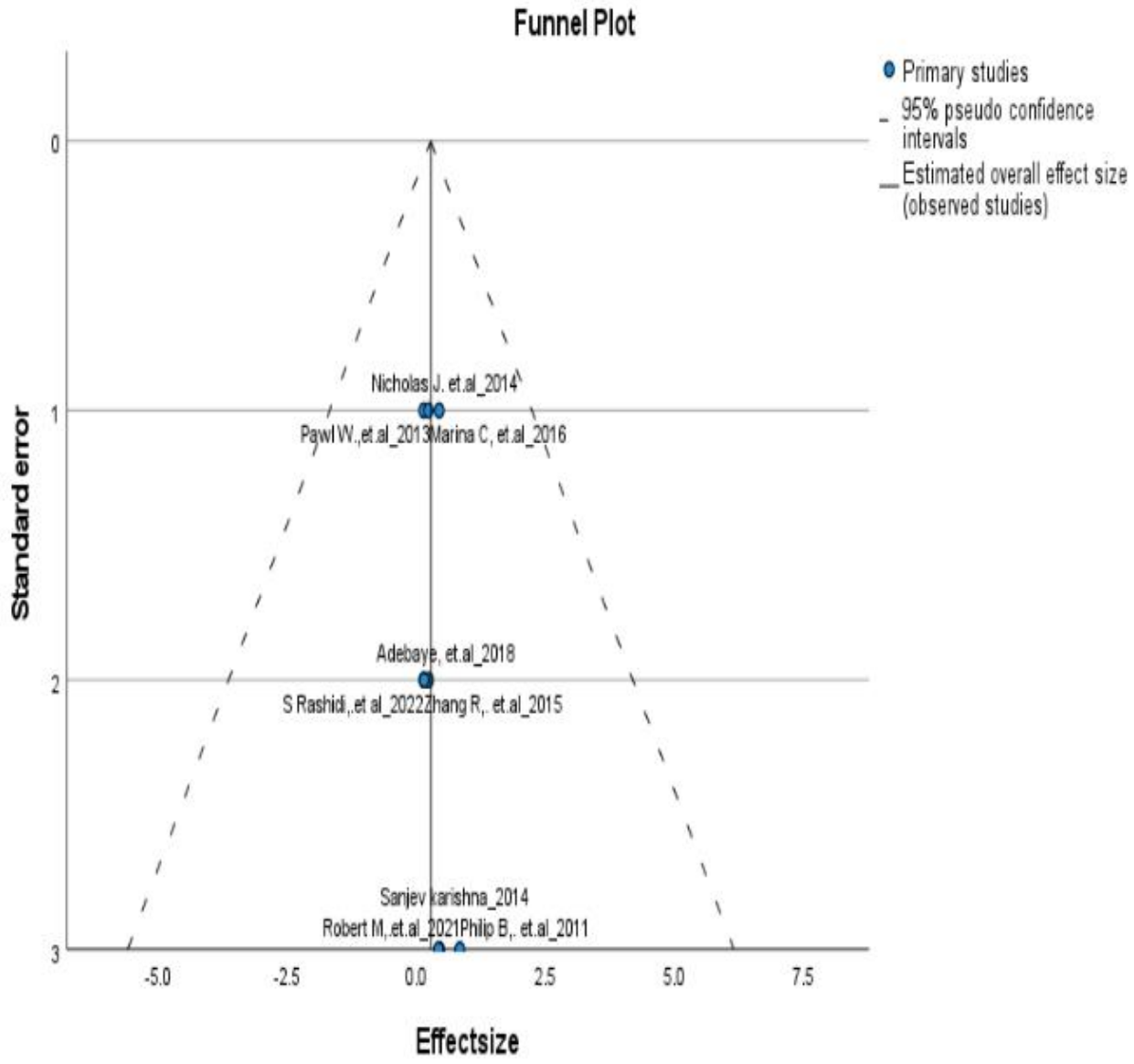

Figure 4. The funnel plot is a scatter plot of treatment effects generated from the novel therapy as reported on the articles. The plot was against a measure of study precision. The funnel plot is a visual tool that shows a systematic heterogeneity from the study. The treatment effect shows a

symmetric inverted funnel shape arising from the data set, in which publication bias is not likely to occur.

The funnel plot in figure 4 above shows less publication bias because most studies are gathered around the summary effect (the line in the centre).

Discussion

The increasing morbidity and mortality rate resulting from the failure of antimalarials has become a big burden to the global health system. This has resulted in more studies being conducted on the new and effective treatment against malaria to reduce the mortality rate of this infectious disease (Shibeshi, Kifle, & Atnafie, 2020). Some of the existing treatments which is being used in many health care systems to treat malaria is Artemisinin and its derivates, the Artemisinin Combination Therapy (ACT) (Salim, Azian, Yusuf, Adyani, & Fuad, 2019). However, recent findings have shown that malaria parasites have developed resistance to these treatments by exhibiting delayed parasite clearance from the host body and resulting in fatal consequences (Heller & Roepe, 2019). In the course of this review, the novel therapies reported include spiroindolone KAE609, Selenium (Se) Compound therapy and RTS, S/S01 antimalaria vaccine (Bejon *et al.*, 2011; White *et al.*, 2014; Adebayo *et al.*, 2018).

Despite the emergence of artemisinin resistance by *P. falciparum*, artemisinins still remain the approved therapy for uncomplicated malaria due to *P. falciparum* (Chavchich et al., 2016). Therefore, sequel to the global concern to improve on the mechanism of action of artemisinins against resistance cases, there is urgent need to develop novel therapy as adjunct to artemisinins or non-ACTs. The rationale for the new drug discovery is to induce dormancy of the malaria parasites in the host's blood and to prevent recrudescence. By so doing, the recurrence situation of malaria will be nipped in the bud. New studies opined the need for the developing of novel drug combination therapy to target different pathways and mechanism of resistance of *P. falciparum* (Schmedes et al., 2018). From the outcome of the present study, Chavchich et al., (2016) reported a temporal dormancy in growth of the early stage of the malaria parasite following ACTs therapy. This effect has been reported earlier by Oduola et al., (2018) as the factor responsible for the recrudescence seen after treatment of *P. falciparum* infections. Thus, this treatment failure resulting from the ACTs gave rise to a new dawn in the resistant malaria treatment. Chavchich et al., (2016), in their study reported a novel and fast-acting antimalarial agent (spiroindolone KAE609) which was reported to have more advantage over ACTs mono treatment as it does not induce temporal growth dormancy seen in the early ring stage of *P. falciparum*. Thus, recrudescence did not occur following the administration of spiroindolone at low concentration. The outcome of the clinical findings from their report revealed that spiroindolone KAE609 was active against the resistance K13 mutant gene of *P. falciparum*. It was also reported that it could be used as an adjunct drug to ACTs with a wide spectrum of activities against resistant parasites.

From the outcome of this present report, it could be opined that some micronutrients play vital roles in the functionalities of the tissues Adebayo et al., (2018). There has been an interest in this area of research because of the roles they play in health by preventing diseases. Selenium is an essential micronutrient which has been reported to increase antioxidant activities of the system because of its glutathione peroxidase content which is needed for a cellular defence mechanism to prevent excess free radicals formation in the body tissues (Achliya et al., 2004). Adebayo et al.,

(2018) in their study posited the use of antioxidants agent in combination with ACT as a novel therapeutic strategy in malaria treatment. The outcome of the report revealed that the co-administration of Selenium and ACT resulted in more efficacies against the malaria parasite when compared to the treatment with only ACT. The effect of selenium will further mop up the free radical species released during post-infection oxidative stress. The outcome of the study is in line with that reported by Vasquez et al., (2021) where ACT was reported to increase the peroxidation level of lipid with a decrease of the antioxidant in the plasma. On the contrary to the co-administration of selenium to ACT as reported in the current review, Kavishe et al., (2017) reported that artemisinins are prodrugs with endoperoxide bridge and act by forming free radicals which in turn inhibit heme polymerization in the malaria parasites. Thus, the study posited that co-administration of the artemisinins with antioxidants can affect the efficacy of the ACTs following a redox reaction between the two moieties.

Another novel therapy developed for the treatment of malaria resistance according to Agnandji et al., (2014) is RTS,S/AS01. According to the report, it has efficacy to protect the host against a spectrum of activities at the various life cycle of the *P. falciparum.* The vaccine was also tested in children aged 5 to 17 months during the first phase of the vaccination at endemic regions. The efficacy of the vaccine was reported to be about 40%. RTS,S/AS01 is the most current and advanced vaccine for malaria infections. It is the first malaria vaccine to undergo wide coverage of clinical trials during the phase III assessment in Africa continent. The pre-erythrocytic stage of *P. falciparum* is the primary target of the RTS,S/AS01 malaria vaccine. As a mechanism of action, it induces cellular and humoral response to the host's immunity. This action, affects the parasite's sporozoites and the schizonts. The outcome of the clinical result of the novel vaccine varied at each setting, but there was no significant difference between the settings. On the contrast, Bejon et al., (2011) reported a decrease in the efficacy during the previous phase II clinical trials. the variation in the vaccine efficacy at different settings as observed during the clinical trial is not associated with differences in the malaria burden of the region. In the outcome of the findings, there are factors that could have contributed to the variation in the vaccine efficacy at different settings, these include but not limited to the incidence of mild anemia at the enrolment stage of the participants, hence, denoting malaria exposure at the individual level which affected the vaccine efficacy negatively (World Health Organization, 2019). In children, there was no correlation seen between the vaccine efficacy and transmission, however, the efficacy is higher at various settings, thus resulting in a lower incidence of malaria. Another factor that could be responsible for the variation of the clinical outcome of the malaria vaccine may be genetic and environmental. The heterogeneity found in the study could be due to differences in participants for the clinical trials, management and severity of the malaria infection of the individuals used for the trial. To reduce the risk of false findings from the systematic review, a practical guideline is needed. This could be achieved by, avoiding statistical evaluation such as subtheme meta-regression and analyses, instead of adopting a 'cautious' approach to the evaluation and interpretation unless availability of a large number of articles. To reduce the heterogeneity of the systematic review findings, it is obvious to carry out a test of statistic for the level of heterogeneity of the outcome from the review. The best approach adopted in this review for heterogeneity was based on a summary statistic from each of the articles. However, the test has been shown that in practice the test statistic has a low degree such that a moderate degree of true heterogeneity may not be revealed.

The resistance conferred by the parasite strain on the antimalarial drug enables the parasite to acquire the ability to multiply and survive notwithstanding the administration and absorption of antimalaria drugs such as the Artemisinin Combination Therapy (Shibeshi, Kifle and Atnafie, 2020). The outcome of the present systematic review which reported some novel therapy that can be used for the treatment of resistance malaria infections is in line with the report from a previous study by Shibeshi et al., (2020) which concurred with the emergence of resistance cases of malaria treatment which some of the factors which facilitate it has been linked to the rate of mutation of the parasite gene conferring the resistance, the total parasite load, the strength and duration of the antimalarial agent, poor compliance to treatment and non-adherence to treatment guideline. However, the novel therapy reported in this review can only work efficiently if some of the predisposing factors that usually lead to resistance are critically considered. Some of these factors that may impede the effectiveness of the novel therapy include, but not limited to fake drugs, improper dosing, and poor pharmacokinetic properties. These factors can lead to insufficient exposure to the drug on the parasites, with such inadequate exposure, the parasite can easily adapt and their genes may mutate to confer resistance on these novel therapies. Low quality and insufficient quantities of this novel antimalarial may aid and abet malaria resistance in the future. Apart from the novel therapy, the efficacy of existing antimalarial can be sustained prolonging the duration of therapy. van der pluijm *et al.*, (2021) opined that a 3-day oral treatment of malaria using artesunate can be followed by a 3-day treatment with ACT class, this has been found to be very effective, Although, the challenge with this therapy is likelihood of poor compliance and adherence as patients may feel quickly better after the first phase.

Conclusion

The increasing morbidity and mortality rate owing to malaria has burdened the global health system. This has resulted in more studies to be conducted on the new and effective treatment against malaria to reduce the mortality rate of this infectious disease. The more recent treatment which was developed and being used in many health care systems to treat the malaria are Artemisinin drug and Artemisinin Combination therapy (ACT). Recent studies show that malaria has developed resistance against treatments and results in the insufficient parasite clearance from the host body and leads towards the fatal consequences. The novel therapies used in this review were spiroindolone KAE609, Se Compound therapy and RTS, S/S01 anti malaria vaccine. It concluded that these novel therapies have significant effectiveness to reduce paracitemia in the host body by targeting the K13 mutant genes, pfrTASe of parasite and targeting the different molecular biological pathways of the *p. falciparum* parasite. In conclusion, these different novel therapies in combination with ACT treatment can be used to treat resistant malaria and reduce the mortality rate.

Ethical aspects and impact on the society

In a systematic review, the ethical considerations are quite dissimilar from those for empirical research (Weingarten, Paul and Leibovici, 2004). The ethical consideration is concerning potential conflict of interest in the trials and publication bias (Suri, 2020). In systematic reviewers,

confidential or sensitive information from participants is not collected, unlike empirical research where such information is collected and thus strict ethical measures are approved by relevant bodies. In systematic reviews, the documents are publicly accessible thus does not require an institutional ethics approval prior to the systematic review. There are no typical guidelines for such research. Nonetheless, in the recent time, it is more inclusive and it now plays a part in policy, practice, and public perception (Suri, 2020). . In the present meta-analysis, ethical considerations of the interests of different authors are well-represented during the interpretation of their research findings. In line with the ethical consideration, the research identified an appropriate epistemological orientation, an appropriate purpose, systematic search for relevant literature based on eligibility, and then, evaluation and interpretation of the selected articles. And finally, constructing connected understandings of the research outcomes to inform decisions.

The present study will offer some benefits geared towards contributing to existing data on malaria resistance updates to the general public and filling the gap as no systematic review has recently reported the novel drugs discovered for the treatment of malaria following the devastating resistance cases reported in recent time which is threatening public health. The outcome of the study will inform the decision making institutions in the health hector on the right directions towards combating malaria resistance. The information provided in the study will guide the society in preventing further resistance problems in the malaria world. With the factors that result these resistances revealed in the study, radical and aggressive sensitization would inform the public on the need to maintain compliance and adherence to malaria treatment. This meta-analytic research will be helpful in the research institutes in dealing with the issues of resistance in malaria. It will further have some impacts in future clinical trials of the novel drugs recruitment, monitoring patients, and treatment goals. On the other hands, the outcome of this finding will help upcoming researcher like students who may have interest in malaria research as it will help the students' access evidence-based results in this area of research. The outcome of the meta-analysis will help the general public especially in remote and underdeveloped nations in Africa continent and beyond to impact positively on the public health issues.

Future perspectives

More clinical studies need to be done in the future to unravel more novel drug discoveries. The scientists are expected to work on the structure-activity relations of the molecules of the novel therapies in order to shoulder high in the issue of malaria parasite resistance. The side effects and interactions are areas of interest. Future studies may look at novel drug interactions with other drugs, food and diseases. More research can be done to discover more therapies to curb the deadly disease, malaria from endemic regions like West African countries and under-developed countries. Among the entire breakthrough in new drug discoveries against the malaria parasite, vaccine development and implementation are the sure solutions to this matter following the antecedents of vaccines in other life-threatening diseases of public health concern Bejon et al., 2011; White et al., 2014; Adebayo et al., 2018). A few decades ago, systematic reviews have evolved to become more methodologically inclusive and play a powerful role in influencing policy and practice, in line with this, further research needs to be conducted to gather public perception on the discovered novel antimalarial drugs. Educational researchers can often draw from the

conceptual traditions of consequentialism and ethical consequentialism. Future research can leverage on the findings from this meta-analysis to undertake a cost-benefit study.

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