# The return of chloroquine susceptible *Plasmodium falciparum* in Sub-Saharan Africa: a protocol for systematic review

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**Protocol**

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# Abstract

**Background:** Following withdrawal and/or restricted use of chloroquine (CQ) as first line malaria treatment in many countries, studies have reported an increased number of CQ susceptible *Plasmodium falciparum* in several countries with subsequent dropping of CQ resistance. Since the future of malaria control and elimination is still uncertain with rising resistance in currently available antimalarials such as artemisinin based combination therapy (ACT), a review on current resistance profile of CQ in *P. falciparum* is of paramount importance.

**Methods**: A systematic search in PubMed/MEDLINE, EMBASE, COCHRANE central, Google Scholar and Web of science will be done. We will consult thesis repositories to identify additional studies and search the websites of key healthcare organizations (World Health Organization (WHO), public health agencies). Similarly, a grey literature search will be done with help of Google. Data from 2000 and onwards published in English will be included and the reporting will be done in accordance with the Preferred Reporting Items for Systematic Reviews and Meta- Analyses Protocol (PRISMA-P).

**Discussion:** This article provides a detailed account of this systematic review protocol that will be used to report the current status of CQ resistance in *P. falciparum* following its restricted use and/ or withdrawal as the first-line treatment of uncomplicated malaria. Mutations in *P. falciparum* chloroquine transporter (*pfcrt)* gene and *P. falciparum* multidrug resistance (*pfmdr1)* gene, predict the clinical outcomes following treatment using CQ. On the other hand, countries with restricted use of CQ observed CQ-susceptible *P. falciparum* reemergence and are now predominant. Subject to its susceptibility profile that will be generated from this review, CQ may still be considered for malaria prevention in some population groups such as children with sickle cell disease and pregnant women. Additionally, CQ may be reintroduced in future, ideally in combination with other antimalarial drugs, especially in areas where disappearance of chloroquine resistance is evident while safe and affordable alternatives antimalarials are limited.

**Systematic review registration**: PROSPERO registration number CRD42020154844


# Background

Malaria is one of the significant global health concerns. In 2017 globally, there were a total of 239 million cases with Africa and South-east Asia being highly burdened contributing 92% and 5% of the cases respectively. Additionally, malaria resulted into a total of 235,000 deaths worldwide, 61% of which were among the under-fives. Despite the global decrease in the incidence rate of malaria from 72 in 2010 to 59 in 2017 per 1000 population, the progress in malaria control and elimination is still slow especially in sub-Saharan Africa (1).

*Plasmodium falciparum* is reported to be the most predominant species causing malaria in almost all malaria endemic regions, accounting for a total of 99.2% of cases in Africa, 62.8% in South east Asia and 69% in Eastern Mediterranean (1). This explains why the mortality rate is high in these regions since *falciparum* species is the most dangerous of all four *Plasmodium* species. Climate is among several

factors that explain the pattern of plasmodium species distribution in these regions and the mosquito *Anopheles gambiae* being the most efficient in malaria transmission, its lifecycle is more favored in tropical climates.

Management of malaria in endemic regions where *P. falciparum* has been reported to be most prevalent has gone through different phases; initially CQ was reported to be the first line antimalarial drug in most of (all) malaria endemic regions prior to development of CQ resistant *P. falciparum* (2,3). CQ use contributed to the great effort in malaria control starting from 1950's (4).

Wide use of CQ especially with presumptive treatment and mass drug administration in most countries is said to have contributed to its resistance starting from late 1950s in South-east Asia and South America and later in Africa, with *pfcrt* (*P. falciparum* chloroquine transporter ) gene mutation being implicated as the biological cause (5–7). Development of CQ resistant species led to the increased malarial transmission as well as morbidity and mortality (8). After the phase out of CQ most countries then adopted the use of sulfonamide-pyrimethamine alone or in combination. Currently, most of malaria endemic regions have included artemisinin based combination therapy (ACT) as the first drug of choice for management of uncomplicated malaria as recommended by WHO since 2007 (9–12).

Following withdrawal of CQ use as the first line antimalarial drug in many countries, studies have reported increasing number of CQ susceptible species in several countries with subsequent dropping of CQ resistance with up to >90% susceptibility were reported (4,13–15). Considering more than a decade of ACT use, currently ACT has been reported to develop resistance both partial and complete in several occasions and a change of malaria treatment policy is indicated if the failure rate is >10% (12). The future of malaria control is still uncertain with such rising ACT resistance, furthermore a review on CQ susceptible *P. falciparum* is of paramount importance. This review will form basis for considering CQ reintroduction in future, ideally in combination with other antimalarial drugs.

# Methods

### Study registration

The present review protocol is being reported in accordance with the reporting guidance provided in the Pre-ferred Reporting Items for Systematic Reviews and Meta- Analyses Protocols (PRISMA-P) (see PRISMA-P checklist in Additional file 1). This review protocol has been registered within the International Prospective Register of Systematic Reviews (PROSPERO database registration number: CRD42020154844).

### Eligibility criteria

### Type of studies and participants

Both clinical trials and observational studies (including cross sectional, cohort and case–control studies) will be included. *P. falciparum* with confirmed chloroquine resistant or susceptible molecular markers.

Several markers associated with *P. falciparum* antimalarial drug resistance have been identified (16). These include encoding chloroquine (CQ) resistance transporter (*pfcrt*) and multidrug resistance transporter-1 (*pfmdr-1*) both located on the food vacuole of the parasite involved in CQ resistance (17). However, the CQ transporter *pfcrt* is a stronger predictor of CQ resistance than *pfmdr1* (18). Single nucleotide polymorphisms of *pfcrt* (K76T) in field isolates correlate with a resistance phenotype in in vitro assays and are sensitive markers for treatment failure in patients (19,20). The single K76 is for susceptible allele, single 76T is a marker for resistant allele while K76/76T (susceptible/resistant) is for mixed alleles (13).

### Types of interventions and outcomes

Countries with restricted use and/or withdrawal of CQ for treatment of malaria infection. Following withdrawal of CQ use as first line malaria treatment, studies have reported increasing number of CQ susceptible species in several countries with subsequent dropping of CQ resistance (4,13,14,21). Invitro prevalence of chloroquine resistant *P. falciparum* and/ or prevalence of chloroquine sensitive *P. falciparum* using *pfcrt* and *pfmdr1* molecular markers.

### Searches

We will systematically search PubMed/MEDLINE, EMBASE, COCHRANE central, Google Scholar and Web of science. We will consult thesis repositories to identify additional studies and search the websites of key healthcare organizations (WHO, public health agencies). Similarly, a grey literature search will be done with help of Google. Data from 2000 and onwards published in English will be included and searches will be re-run prior to the final analysis.

### URL to search strategy

We will develop a rigorous systematic search strategy with a health sciences librarian who has systematic review experience using published guidelines of the Cochrane Collaboration. The strategy will be developed for PubMed/MEDLINE (Additional File 2) and EMBASE using keywords and MeSH (MEDLINE) or EMTREE (EMBASE). To be as inclusive as possible, we will limit the search strategy to terms covering the susceptible OR chloroquine resistant *P. falciparum.* Keywords such as *pfcrt*, *pfmdr1*, chloroquine, resistant, susceptible and *P. falciparum* will be used. This search strategy will also be adapted to the other databases.

### Data extraction (selection and coding)

**Study selection:** Study selection will be managed using Covidence software (Australia) where two independent reviewers will evaluate articles for potential inclusion by screening titles and abstracts and will assess full publications to determine eligibility for final inclusion. Between each assessment, results will be discussed to reach a consensus on the interpretation of inclusion criteria. Any further disagreement on study eligibility will be resolved by consensus, and a third reviewer will be consulted if necessary. If information on eligibility is unavailable and/ or unclear, study authors will be contacted to

clarify. Duplicate publications will be identified and removed using reference manager (Endnote X9, USA). Identified publication(s) will be analyzed using criteria based on most recent dates, largest sample size, maximum correspondence with inclusion criteria and minimal risk of bias.

**Data coding**: Data that will be extracted from study documents, including information about study design and methodology, participant demographics and baseline characteristics, prevalence of chloroquine resistant/ susceptible etc (Additional File 3). Unavailable, unclear information and/or additional details will be requested from the study investigators. Data will be recorded using excel spreadsheet and Systematic Review Data Repository-Plus.

### Risk of bias (quality) assessment

Risk of bias in observational studies will be evaluated using the ROBINS-I risk of bias assessment tool for non-randomized studies. The tool evaluates baseline and time-varying confounding, co-interventions, selection bias, classification bias (intervention), missing data, and bias in outcome measurement. While for randomized controlled trials risk of bias will be evaluated using the Cochrane risk of bias tool. Two reviewers will independently evaluate risk of bias and rate studies using respective tools (low, moderate serious, critical, unclear for the ROBINS-I tool and high, low, unclear for the Cochrane risk of bias tool). Disagreement will be resolved using arbitration by a third reviewer

### Strategy for data synthesis

Depending on data availability the trend of chloroquine resistance will be sorted per year based on change of chloroquine treatment. For heterogeneity across studies in terms of populations, design, methods, intervention, and/or outcome (s) will be presented using a systematic narrative synthesis. We will explore the results according to categories of interventions and outcomes taking account of risk of bias, in line with Centre for Reviews and Dissemination recommendations. The narrative will be written by the lead reviewer and then checked independently by at least one other reviewer. A third reviewer will adjudicate any disagreements. We will measure heterogeneity for each meta-analysis using $I^2$.

### Analysis of subgroups or subsets

When data available the trend of chloroquine resistance will be sorted based on year of abandonment in every country.

## Discussion

This article provides a detailed account of this systematic review protocol that will be used to report the current status of CQ resistance in *P. falciparum* following its restricted and/ or withdrawal as the first-line antimalarial drug for treatment of uncomplicated malaria.

Gene mutations in *pfcrt* and *pfmdr1* as a result of pressure use of CQ, predicts CQ resistance of *P. falciparum* malaria predict the clinical outcomes following the treatment using CQ (22). *Pfmdr1*

mutations do contribute to treatment failure, but they provide an assistance to *pfcrt* mutations to exert an effect on the response to chloroquine. However, persistence of *pfmdr1* mutations in a population with a very low prevalence of *pfcrt* mutations would not be expected to reduce CQ efficacy in that population.

On the other hand, countries with restricted used of CQ observed CQ-susceptible *P. falciparum* parasites have reemerged and are now predominant (4,13,14,23). In contrary to this, high levels of CQ resistance have persisted in countries with unrestricted use of CQ, i.e., Nigeria (24). This observation suggests that continual circulation of CQ in the study area increase spread of CQ-resistant *P. falciparum* parasites (24). The suggested cause of high levels of CQ resistance in Nigeria concurred with an evidence which documented that, the withdrawal of antimicrobial drug pressure does not always compromise the fitness of resistant microorganisms and result in selection of drug-sensitive phenotypes (25).

Lastly, after the ban of CQ use in treatment of uncomplicated malaria, the drug was still recommended for use as chemoprophylaxis against malaria in sickle cell disease patients in sub-Saharan countries including Tanzania (26) but pressure from the drug resistance stopped its use as chemoprophylaxis (27). Subject to its susceptibility profile that will be generated from this review, chloroquine may still be considered for malaria prevention, i.e. sickle cell disease children, intermittent preventive therapy in pregnancy women or the reintroduction in future, ideally in combination with other antimalarial drugs (28), especially in areas where disappearance of chloroquine resistance is evident while safe and affordable alternatives antimalarials are limited.

# Abbreviations

ACT: Artimesinin based combination therapy

CQ: Chloroquine

*pfcrt*: *Plasmodium falciparum* chloroquine transporter

*pfmdr1*: *Plasmodium falciparum*

PRISMA-P: Preferred Reporting Items for Systematic Reviews and Meta-Analyses Protocols

PROSPERO: Prospective Register of Systematic Reviews

WHO: World Health Organization

# Declarations

### Ethics approval and consent to participate

No ethics approval is required for this systematic review because information will be extracted from published studies (secondary sources).

### Consent for publication

Not applicable.

### Availability of data and materials

Not applicable.

### Competing interests

The authors declare that they have no competing interests.


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### Authors' contributions

GMB and BJN designed the study protocol and drafted the manuscript. TM, AT, RM and DS revised the manuscript. All authors have read and approved the final version of this manuscript


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