Drug resistance markers within an evolving efficacy of anti-malarial drugs in Cameroon: a systematic review and meta-analysis protocol

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Protocol

Keywords: Malaria, anti-malarial, resistance, mutations, systematic review, meta-analysis, Plasmodium falciparum, Cameroon

Posted Date: May 14th, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-26096/v1>

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Abstract

Background Cameroon remains a country faced with high malaria burden despite enormous efforts made in the control of the disease. The rapid development and dispersal of mutations associated with anti-malarial drug resistance influenced policy changes from the use of chloroquine, amodiaquine and sulphadoxine-pyrimethamine to the adoption of artemisinin-based combination therapies (ACTs) for the treatment of uncomplicated falciparum malaria. Different studies have identified the frequency of key markers in *Plasmodium falciparum* associated with drug resistance without a clear picture on the localisation of potential hotspots that may drive the emergence of resistance to the currently used ACTs. This systematic review and meta-analysis aims to determine the prevalence and distribution of *P. falciparum* drug resistance markers within an evolving efficacy of anti-malarial drugs in Cameroon from 1990 to present.

Methods The PRISMA, PRISMA-P and STREGA statements will be adopted in the quality assessments of studies to be included in this review. The electronic databases of Medline via Pubmed, Google Scholar and Science Direct will be searched by two independent researchers using different MeSH terms and Boolean operators (AND, OR). More so, unpublished data that will be sourced from academic libraries will also be extracted. Quantitative syntheses will be done using the “metaphor” and “meta” commands in the R statistical software package version 3.5.2. Heterogeneity will be assessed using Cochran Q and the I^2 . The random effect model will be used as benchmark to combine studies showing heterogeneity.

Discussion The primary outcome of this review is to identify and describe molecular markers conferring drug resistance in *Plasmodium falciparum* parasites that have been circulating for a period of over 30 years in Cameroon. This review will be able to pool data from previously published and unpublished studies on anti-malarial drug resistance gene mutations. This will provide evidence to support the continuous use of ACTs in the treatment of uncomplicated *P. falciparum* malaria. Moreover, it is also hoped that potential hotspots driving the emergence and spread of anti-malarial resistance markers will be identified.

Systematic review registration: PROSPERO submission identification number is 162620

Background

According to the WHO global statistics, malaria accounted for 228 million cases and 405,000 related deaths in 2018 [1]. Cameroon remains a country with a high malaria burden and impact despite enormous efforts made in the control of the disease [1]. In Cameroon, the rapid emergence and dispersal of drug resistance was responsible for the change of chloroquine (CQ) use as the first-line therapy for treatment of uncomplicated *Plasmodium falciparum* malaria in 2002 and later amodiaquine (AQ) monotherapy and sulphadoxine-pyrimethamine (SP) between 2002 and 2004 [2]. A major drug policy change was recorded in 2004 when the government of Cameroon officially aligned with WHO recommendations by adopting artesunate-amodiaquine (ASAQ) and later artemether-lumefantrine (AL) in

2006 as first-line treatments for uncomplicated malaria [2, 3]. ASAQ and AL drugs are distributed in the proportions of 75% and 25% respectively in public health facilities [4]. It is important to note that, the AL combination is relatively predominant within the private health facilities and vendors [4]. The efficacy of anti-malarial drugs is linked to the presence or absence of parasites resistant to these drugs in the population. Thus, regular monitoring of drug resistance markers is very essential to malaria control programmes in endemic regions. The use of advanced molecular biology techniques has greatly facilitated the identification of key amino acid changes in the genes of *Plasmodium falciparum* chloroquine resistant transporter-*Pfcr*t (C72S, V73K, M74I, N75E, K76T, A220S, Q271E, N326S, I356T, R371I), *Plasmodium falciparum* multi-drug resistant 1-*Pfmdr*1 (N86Y, Y184F, S1034C, N1042D, D1246Y, copy number variation), *Plasmodium falciparum* dihydrofolate reductase-*Pfdhfr* (A16V, C50R, N51I, C59R, S108N/T) and *Plasmodium falciparum* dihydropteroate synthase-*Pfdhps* (I431V, S436A/F, A437G, K540E/N, A581G, A613S/T) associated with resistance to different anti-malarial drugs [5–9]. Between 2009 and 2010, single nucleotide polymorphisms in the *Pfk13* propeller domain from Cambodia isolates excluding those from Tanzania were associated with delayed parasite clearance of artemisinins [10]. The epicentres of artemisinin resistance are countries located within the Greater Mekong sub-region (GMS) namely, Cambodia, China (Yunnan Province), Lao People's Democratic Republic, Myanmar, Thailand and Vietnam [11]. There are currently about 200 non-synonymous mutations in the K13 gene that have been identified and reported [10–14]. Moreover, a total of 9 *Pfk13* non-synonymous single nucleotide polymorphisms (F446I, N458Y, Y493H, R539T, I543T, P553L, R561H, and C580Y) are associated with delayed parasite clearance [10–14]. The F446I, R539T, I543T, P574L and C580Y mutations are the most common and have the highest occurrences [10–14]. In Africa, the *Pfk13* mutation with the highest geographical distribution is A578S [11–14] and the presence of R561H mutation has recently been reported in Tanzania [15]. Even though that artemisinin resistance has not been widely reported in Africa, there is need for continuous surveillance in order to avoid a scenario similar to what happened in the past with the chloroquine, amodiaquine, and sulphadoxine-pyrimethamine. In Cameroon a number of reviews carried out in the past did not present sufficient data on the frequency and distribution of anti-malarial drug resistance gene polymorphisms in Cameroon [13, 16, 17]. This systematic review and meta-analysis aims to determine the prevalence and distribution of *P. falciparum* drug resistance markers within an evolving efficacy of anti-malarial drugs in Cameroon from 1990 to present.

Methods/design

Review question

What is the reported prevalence of *P. falciparum* drug resistance markers before and after the adoption of artemisinin-based combination therapies in Cameroon?

Objectives of the systematic review

This review aims to:

1. Determine the prevalence of anti-malarial drug resistance markers in Cameroon from 1990 to present. The key *Plasmodium falciparum* anti-malarial resistance markers shall include: chloroquine resistance transporter (*Pfcr1*), multi-drug resistance 1 (*Pfmdr1*), dihydrofolate reductase (*Pfdhfr*), dihydropteroate synthase (*Pfdhps*), atpase 6 (*Pfatp6*), cytochrome b (*Pfcyb*) and kelch 13 (*Pfk13*).
2. Evaluate the relationship between the efficacy of ACTs (ASAQ and AL) and prevalence of *falciparum* drug resistance mutations (chloroquine resistance transporter mutation 76T and multidrug resistance 1 mutation 86Y).
3. Investigate the impact of the amount of artesunate-amodiaquine (ASAQ), artemether-lumefantrine (AL) and sulphadoxine-pyrimethamine deployed to the different Regions on the evolution of drug resistance markers in Cameroon.

Registration of the systematic review protocol

The review protocol has been submitted to the International Prospective Register of Systematic Reviews (PROSPERO) website with identification number 162620.

Search strategy

An electronic systematic strategy based on the combination of key words will be used to search articles from Medline via Pubmed, Google Scholar, and Science Direct databases. Both interventional and observational studies will be retrieved to be included in the review. The following MeSH search terms will be combined using the Boolean operators "OR" and "AND": "anti-malarial", "drug resistance", "*Pfcr1*", "*Pfmdr1*", "*Pfdhfr*", "*Pfdhps*", "*Pfatp6*", "*Pfcyb*", "*Pfk13*", "mutations", "gene polymorphisms", "amino acid changes", "*Plasmodium falciparum*", "Cameroon".

Additional searches

The reference lists of published articles will be searched for eligible studies. Authors will be contacted in the case whereby full length articles could not be assessed. Malaria annual reports will be obtained from the Cameroon National Malaria Control Programme (NMCP) and Ministry of Public Health. In addition to published studies, unpublished MD, MSc and PhD theses will be accessed for inclusion in the study.

Eligibility criteria

Inclusion criteria

The systematic review and meta-analysis will include the following type of studies:

i) studies published from January 1990 to present; ii) studies on human participants of all ages; iii) original articles of studies that investigated either asymptomatic, uncomplicated or severe *Plasmodium falciparum*; iv) studies in which PCR genotyping of anti-malarial drug molecular resistance markers (*Pfcr*, *Pfmdr1*, *Pfdhfr*, *Pfdhps*, *Pficyt*, *Pfatp6*, *Pfk13*) were done; v) studies written in English or French; vi) studies done within Cameroon; and vii) all multi-centric studies in which Cameroon was one of the sites or cases imported malaria from Cameroon were included in this The selection and inclusion of studies will be done according the population intervention comparator outcome (PICO) format.

Non-inclusion criteria

The following types of studies will not be included: i) abstracts; ii) studies on in vitro, ex vivo and in vivo anti-malarial drug resistance without DNA sequence genotyping; iii) genetic association studies on *Pfcr* gene; iv) studies on genetic diversity and population structure of *Plasmodium falciparum* without drug resistance; and v) studies on diagnostic accuracy of methods for detection of *P. falciparum* and mixed *Plasmodium* species infections.

Review process

Articles identified from searches of the computerised databases will be screened for eligibility based on title and abstract. Ineligible articles and duplicates will eventually be removed. Full-length articles of the selected studies will be read to confirm for fulfilling of the inclusion criteria before data extraction began. Two independent reviewers (PTNN and LNN) will screen the titles and abstracts to identify potentially eligible studies and data from full-length articles that fulfil the inclusion criteria will be extracted. Discrepancies will be resolved by mutual consent or by independent review from the third researcher (AMN). The whole process will be supervised by WFM and MA.

Data extraction procedure

Data extraction process will focus on the types of study design (observational versus interventional), year the studies were conducted, study site, sample size, age of participant, genotyping method, genotyping accuracy, drug resistance gene, type of amino acid changes, and prevalence of mutations (Additional file 1). Mixed genotypes will be considered as mutants during data collation on frequency of mutations

derived from different studies. The Microsoft Excel 2010 shall be used to design the data extraction sheet. The database in Microsoft Excel will be piloted and validated before completion of the review process. **Methodological quality assessment and protocol development**

The PRISMA [18, 19], PRISMA-Abstracts [20], PRISMA-P [21, 22] and STREGA [23] statements will be applied in the development of the protocol for this review and meta-analysis.

Data management

Data will be managed in the Zotero standalone software version 5.0.56. Eligible articles will be imported into the software and duplicates removed.

Data analysis, heterogeneity assessment and data interpretation

Quantitative syntheses will be done using the “metaphor” and “meta” commands in the R statistical software package version 3.5.2. The heterogeneity of the included studies will be evaluated using Cochran Q and the I^2 statistics. The random effect model will be used as benchmark to combine studies showing heterogeneity of Cochran Q with $P < 0.10$ and $I^2 > 50$ at 95% confidence interval [24].

Heterogeneity to be assessed with I^2 will be classified into three categories as follows: low (0-49%), moderate (50-74%) and high (75-100%). Data derived from an article published by one author or same authors in a particular year will be merged before presentation on forest plots. Forest plots will be used to present the data on pool prevalence of mutations in anti-malarial drug resistance genes. Sub-group analyses shall be also done to demonstrate the aggregate prevalence rates of *Pfcr*t, *Pfmdr*1, *Pfdhfr*, *Pfdhps* and *Pfk13* gene mutations. Trend analyses on bar charts will done to demonstrate the evolution of key anti-malarial drug resistance markers and quantities of artesunate-amodiaquine, artemether-lumefantrine and sulphadoxine-pyrimethamine deployed to the different regions in Cameroon over the years. The Pearson Chi square test in the IBM SPSS version 20.0 software package will be used to establish the evolution of drug resistance markers over time. The Pearson correlation coefficient (r) will be used to assess the relationship between efficacy of ACTs (AL and ASAQ) and prevalence of *Pfcr*t 76T and *Pfmdr*1 86Y mutants over time. The level of significance will be set at $p < 0.05$ at 95 % confidence interval. Furthermore, the relationship between the efficacy of ACTs (ASAQ and AL) and anti-malarial drug resistance makers (*Pfcr*t 76T and *Pfmdr*1 86Y) will be assessed on plots.

Publication bias

Publication bias shall be assessed by using the funnel plot and the Egger statistical test. The funnel plot contains the standard error on the y-axis and proportion on the x-axis.

Discussion

The primary outcome of this review is to identify and describe molecular markers conferring drug resistance in *P. falciparum* parasites that have been circulating for a period of over 3 decades in Cameroon. This review will be able to pool data from previously published and unpublished studies on anti-malarial drug resistance gene mutations. This will provide evidence to support the continuous use of ACTs in the treatment of uncomplicated *P. falciparum* malaria. Moreover, it is also hoped that potential hotspots driving the emergence and spread of anti-malarial resistance markers will be identified. Furthermore, the data generated from this review will provide baseline information on the design and adoption of a robust anti-malarial drug resistance surveillance system nationwide.

Abbreviations

ACT: Artemisinin-based combination therapy, AL: Artemether-lumefantrine, ASAQ: Artesunate-amodiaquine, NMCP: National Malaria Control Programme, Pfcrt: *Plasmodium falciparum* chloroquine resistance transporter, *Pfmdr1*: *Plasmodium falciparum* multi-drug resistance 1, *Pfdhfr*: *Plasmodium falciparum* dihydrofolate reductase, *Pfdhps*: *Plasmodium falciparum* dihydropteroate synthase, *Pfcytb*: *Plasmodium falciparum* cytochrome b, *Pfatp6*: *Plasmodium falciparum* atpase 6, *Pfk13*: *Plasmodium falciparum* kelch 13, PRISMA: Preferred reporting items for systematic reviews and meta-analyses, PRISMA-P: Preferred reporting items for systematic reviews and meta-analyses protocol, SP: Sulphadoxine-pyrimethamine, SPAQ: Sulphadoxine-pyrimethamine-amodiaquine, STREGA: Strengthening the reporting of genetic association studies, STROBE: Strengthening the reporting of observational studies in epidemiology, WHO: World Health Organisation

Declarations

Ethics approval and consent to participate

Not applicable since it is a systematic review and meta-analysis.

Consent for publication

Not applicable.

Availability of data and materials

Not applicable as this is still a systematic review and meta-analysis protocol with no data available for publication.

Competing interests

The authors declare that they have no competing interests.

Funding

WFM, AMN, IMA and PTNN are supported by the MARCAD Consortium through the DELTAS Africa Initiative [grant # DEL-15-010] to the University of Yaounde I. The DELTAS Africa Initiative is an independent funding scheme of the African Academy of Sciences (AAS)'s Alliance for Accelerating Excellence in Science in Africa (AESA) and supported by the New Partnership for Africa's Development Planning and Coordinating Agency (NEPAD Agency) with funding from the Wellcome Trust [grant # 107741/A/15/Z] and the UK government.

Authors' contributions

WFM conceived the research and coordinated the study. AMN and PTNN drafted the manuscript, critically reviewed the manuscript, and wrote the final manuscript. The authors WFM, MA, MSE, IMA, PMN, RN, MNM, LNN, OEN, FAA, CMM, DAF, BAT, OAA, RD, JPC, JDB, CEEM, AA, EA, ET, RGFL, AT and PR proof read the manuscript. All authors read and approved the final manuscript.

Acknowledgements

Not applicable.

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