Artemisinin-based combination therapy (ACT) and drug resistance molecular markers: A systematic review of clinical studies from two malaria endemic regions – India and sub-Saharan Africa

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A B S T R A C T

Artemisinin-based combination therapies (ACT) are currently used as a first-line malaria therapy in endemic countries worldwide. This systematic review aims at presenting the current scenario of drug resistance molecular markers, either selected or involved in treatment failures (TF) during in vivo ACT efficacy studies from sub-Saharan Africa (sSA) and India. Eight electronic databases were comprehensively used to search relevant articles and finally a total of 28 studies were included in the review, 21 from sSA and seven from India. On analysis, Artemether + lumefantrine (AL) and artesunate + sulfadoxine-pyrimethamine (AS + SP) are the main ACT in African and Indian regions with a 28-day efficacy range of 54.3–100% for AL and 63–100% for AS + SP respectively. It was observed that mutations in the Pfcrt (76T), Pfdhfr (51I, 59R, 108N), Pfdhps (437G) and Pfdhr1 (86Y, 184F, 1246Y) genes were involved in TF, which varied with respect to ACTs. Based on studies that have genotyped the Pk13 gene, the reported TF cases, were mainly linked with mutations in genes associated with resistance to ACT partner drugs; indicating that the protection of the partner drug efficacy is crucial for maintaining the efficacy of ACT. This review reveals that ACT are largely efficacious in India and sSA despite the fact that some clinical efficacy and epidemiological studies have reported some validated mutations (i.e., 476I, 539T and 561H) in circulation in these two regions. Also, the role of Pfk13 mutations for emergence of artemisinin (ART) resistance in these countries.

1. Introduction

Malaria is an infectious disease caused in humans by Plasmodium parasites transmitted through bites of infected female Anopheles mosquitoes during their blood meal (White et al., 2014). Plasmodium falciparum and Plasmodium vivax are the main human species involved in malaria cases and deaths, causing approximately 229 million cases and 409,000 deaths worldwide in 2019 (WHO, 2020). P. falciparum is the most dangerous between the two malarial species with an enormous threat to the public health in several endemic countries (WHO, 2020).

Early diagnosis and prompt treatment with effective antimalarial drugs are the two key components of malaria control and elimination (WHO, 2018a). Since the discovery of artemisinin (ART) by Professor Youyou, these medicines have continuously gained importance as an alternate drug, for their high efficacy level against malaria parasites as there has been increased prevalence of resistance to older antimalarial drugs (chloroquine-CQ, sulfadoxine-pyrimethamine-SP) reported from different areas (Blasco et al., 2017; Tu, 2015). The malaria therapy currently includes ACT as first- and second-line treatment in most endemic countries which consists of ART combination (or one of its derivatives) with other drug (s) (or two other antimalarials) referred to as partner drugs (WHO, 2018a). The drug combinations in the ACT not only have different mechanism leading to their improved efficacy but also, the chances of emergence of drug resistance to each component drugs are lower (Ashley et al., 2018). Six ACT are currently recommended by the World Health Organization (WHO) for treating malaria

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cases worldwide: i) artesunate + amodiaquine (AS + AQ), ii) artesunate + lumefantrine (AL), iii) artesunate + sulfadoxine-pyrimethamine (AS + SP), iv) artesunate + mefloquine (AS + MQ), v) artesunate + pyronaridine (AS + PY) and vi) dihydroartemisinin + piperaquine (DHA + PPQ) (WHO, 2019). These different ACT are an essential part of the treatment policies in several malaria endemic countries (Bennett et al., 2017; Recht et al., 2017).

_Plasmodium falciparum_ has developed resistance mechanisms to most antimalarial drugs in vogue including the current ACT where the only known and validated ART resistance foci are the Greater Mekong subregion (GMS) in Southeast Asia (Antony and Parajia, 2016; Menard and Dondorp, 2017). The emergence and spread of ACT resistant malaria parasites have strongly hindered the control efforts in this area and have led initially to increased morbidity and mortality (Chokkajorn, 2018). The appearance and/or spread of malaria parasites resistant to ACT in highly malaria burdened parts of the world such as sSA countries and India are a major concern for the scientist community, along with all the stakeholders in the fight against malaria.

The WHO advocates total compliance of the malaria treatment policy by clinicians, program officers and patients in order to prevent the emergence and propagation of ART resistance (WHO, 2018a). With this view, as an essential part of the Global technical strategy (GTS) for malaria 2016–2030, WHO has outlined the need for a continuous monitoring of ACT efficacy through surveillance systems where one of the main targets in the GTS report is the elimination of malaria in 20–30 countries (which were endemic in 2015) by 2025–2030 (WHO, 2016). The evaluation of therapeutic efficacy is the reference method for monitoring the efficacy of antimalarial drugs and thereafter informing national malaria program for revisions in the treatment policy. The prevalence of polymorphisms in molecular markers associated with drug resistance can be analyzed in parallel to support findings from clinical studies (Maji, 2018).

Some reviews published earlier have focused on either the efficacy of ACT or the prevalence of polymorphisms in drug resistance molecular markers and only one reported study from Africa has both of these aspects but there are no reports yet from India (Adam et al., 2018; Conrad and Rosenthal, 2019; Gogtay et al., 2013; Menard and Dondorp, 2017; Price et al., 2014; Prosser et al., 2018; Whegang et al., 2019). In the present review, the current knowledge on antimalarial drug resistance and ART resistance is briefly summarized (WHO, 2018a). In addition, we conducted a systematic review (SR) to summarize findings from existing literature on the current scenario of molecular markers which were either selected or involved in treatment failures after ACT-based treatment. Finally, a comparative analysis of these findings between sSA countries and India is presented and discussed in this review.

### 2. Antimalarial drug resistance: definition, genetic basis and molecular markers

Antimalarial drug resistance is one of the greatest threat to the control and elimination of malaria globally (WHO, 2018a). The drug resistance is defined as the ability of a parasite strain to survive and/or increase in doses equal to or higher than those usually recommended but within the tolerance of the subject (WHO, 1967). Resistance to antimalarial drugs has only been documented in _P. falciparum_ and _P. vivax_ and the major mechanism responsible for acquisition of this phenotype is due to the mutations in genes associated with drug resistance or increase in their gene copy number (Menard and Dondorp, 2017). _P. falciparum_ resistance has been demonstrated to most classes of antimalarial drugs including quinolines, antifolate and ART derivatives while in _P. vivax_ resistance has been documented for CQ, primaquine and antifolate drugs in several endemic countries (Bansal et al., 2017; Haldar et al., 2018; Price et al., 2014). Regarding _P. falciparum_, the drug resistance-associated genes include chloroquine resistance transporter (_PfCRT_), Pf multidrug resistance protein 1 (_Pfmdr1_), Pf dihydrofolate reductase (_Pfdhfr_), Pf dihydropteroate synthase (_Pfdhps_), Pf cytochrome B (_Pfcbt_) and Pf sodium–hydrogen exchanger gene (_Pfne-1_) (Table 1) (Antony and Parajia, 2016; Menard and Dondorp, 2017).

The well-known single nucleotide polymorphisms (SNPs) associated with different drug resistance-associated genes are 76T in _Pfcrt_, 51I, 59R and 108N in _Pfdhfr_, 437G and 540E in _Pfdhps_; 268N/S/C in _Pfcbt_, 86Y, 184Y and 1246Y in _Pfmdr1_ for _P. falciparum_ (Table 1) (Adjekukor, 2018; Korinszczky et al., 2000; Peterson et al., 1988; Pickard et al., 2003; Singh Sidhu et al., 2002; Triglia et al., 1997). A recent review on the countries in the horn of Africa, revealed a high prevalence of _Pfct_ mutations while mutations in _Pfmdr1_ and _PfATPase6_ genes were low in Ethiopia, Somalia, Djibouti and Eritrea (Jalei et al., 2018). Another report by Berzosa and colleagues showed a prevalence of 78% and 2% of _Pfdhfr_ triple mutants (511/59R/108N) and _Pfmdr1_ double mutants (86Y + 1246Y) respectively in Equatorial Guinea, a Central African country (Berzosa et al., 2017). The _Pfкт_ K76T mutations have been reported from Eastern regions of India, especially from Arunachal Pradesh and Assam, along with mutations in the _Pfmdr1_ gene at the positions 86Y and 1246Y associated with reduced sensitivity to CQ (Das et al., 2014; Sharma et al., 2016). _Pfdhfr_ gene, mutations at codons 16V, 51I, 59R, 108N and 164L and in _Pfdhps_ mutations at codons 436A, 437G, 540E, 581G and 613S/T are reported to be associated with resistance to SP in Arunachal Pradesh, Assam and several other areas too (Kumar et al., 2015; Patel et al., 2017).

### 3. Artemisinin resistance: mechanism, molecular markers and current scenario in sub-Saharan Africa and India

The first case of therapeutic failure with artesunate monotherapy was documented from Thailand-Cambodia border (Dondorp et al., 2009; Noedl et al., 2008) and there are also other studies which have observed delayed parasite clearance and increased recrudescence rates reported treatment with ACT (Witkowski et al., 2010, 2013). Two mechanisms viz. parasitic quiescence and altered temporal response were shown to be responsible for ART resistance using in vitro studies initially and later confirmed by in vivo studies (Klions et al., 2013; Paloue et al., 2016; Witkowski et al., 2010, 2013). The basic mechanism of ART resistance was explained by point mutations in the beta-propeller domain of the gene encoding the Kelch protein 13- _Pfk13_ probably responsible for reduced susceptibility (i.e., delayed parasite clearance time) to ART and its derivatives (Arrey et al., 2014; Menard and Dondorp, 2017; Ouij et al., 2018; Straimer et al., 2015).

The _Pfk13_ gene is located on the chromosome 13 with only one exon and encodes the _kelch_ protein that has three domains namely _Plasmodium_ -specific domain, BTB/POZ domain (Broad-Complex, Tramtrack and Bric a brac; Poxvirus and zinc finger respectively) and a C-terminal six-blade propeller domain (Pasupureddy et al., 2019). A summary of validated and putative mutations in the _Pfk13_ gene associated with ART resistance has been reported (Pasupureddy et al., 2019; WHO, 2018b).

The resistance to ART and its derivatives is confirmed in the GMS, consisting of six countries - Cambodia, Thailand, Myanmar, Lao People’s Democratic Republic, Vietnam and China where the prevalence of mutations in the reported gene is reported to be relatively high (WHO, 2018a). Ikeda and colleagues reported a novel mutation in the _Pfk13_ gene (675V) in Uganda which is currently categorized by the WHO as “associated/candidate” mutations (Ikeda et al., 2018). From sSA, validated mutations (i.e., 561H) in the _Pfk13_ gene responsible for ART resistance as well as some non-synonymous with few novel mutations have been reported (Ikeda et al., 2018; Lu et al., 2017; Menard et al., 2016; Uwimana et al., 2020). In India, mutation at 561H of the _Pfk13_ gene currently validated by the WHO for ART resistance was reported from only one sample (Mishra et al., 2015) (Table 1).
4. Selection and implications of molecular markers in failure treatment after ACT treatment in India and sSA

4.1. Methods

4.1.1. Electronic databases and search strategy

The authors AA, LPKF and SC did comprehensive searching of eight electronic databases including PubMed, EMBASE, MEDLINE, Wiley, ResearchGate, African Journal Online, ScienceDirect and Scopus to identify all relevant papers for the present study. The search for potentially relevant articles was performed from May to July 2019. The main key terms were: “malaria”, “Plasmodium”, “Plasmodium falciparum”, “artemisinin-based combination therapies”, “artesunate “artemether”, “efficacy”, “in vivo”, “molecular markers”, “resistance”, “selection”, “selected”, “treatment”, “treatment failure”, “Africa”, “India”. The names of sSA countries or Indian states were also used in combination with other key terms. These search terms were used in English and French in combination with Boolean operator “AND”, “OR” and truncation element “*”. The search strategy was adjusted according to the requirements of different databases (Supplemental material 1). A summary of the strategy of searching as outlined previously (Supplemental material 2 and 3) (Moher et al., 2009).

4.1.2. Screening strategy of papers retrieved from the electronic databases

Titles and abstracts of papers were independently reviewed by authors AA, LPKF and SC using the above mentioned search strategy. The full texts of potentially eligible publications were directly retrieved from databases if titles or abstracts were informative (i.e., giving all key terms useful to include or exclude a paper in the review). If not, the full text was retrieved and totally read in order to decide if the papers fulfilled the inclusion criteria. The publications not available free online were either purchased or requested from the corresponding author. The reference lists of relevant papers were also examined.

4.1.3. Eligibility criteria of studies included in the review

The studies included in the present study were considered only after they fulfilled the following four prerequisites: (i) Treatment efficacy studies (TES) having addressed either the effect of ACT on the selection of molecular markers associated with drug resistance or the involvement of these markers associated in treatment failure cases, (ii) studies conducted in India and sSA countries, (iii) articles written in English or French languages and (iv) articles peer-reviewed and published between January 2008 and December 2018 (a 11-year period was chosen in order to reduce the methodological variability between eligible studies. In addition, by the year 2008 ACT were already adopted and made available at affordable prices for treatment of all ages in public sector in most malaria endemic countries). All discrepancies between the authors AA, LPKF and SC were solved in discussions with the supervising author (VS).

4.1.4. Bias risk assessment

The methodological quality of studies was assessed using the Joanna Briggs Institute (JBI) critical appraisal tools for use in JBI systematic reviews checklist for randomized controlled trials available at: https://joannabriggs.org/critical-appraisal-tools (The Joanna Briggs Institute, 2020). This tool consists of 13 questions addressing different type of bias (selection, performance, attrition and reporting) related to different methodological aspect of the included studies (i.e., randomization process, blinding). The bias risk for each methodological element was classified as “high”, “low” and “unclear”. Quality of studies was independently evaluated and any disagreement was resolved through discussion.

4.1.5. Data extraction and verification for consistency

The collected information from the studies included the following: the first author name, the year of publication, the name of the African country or Indian state, the year of data collection, the evaluated ACT, the study population, the level of malarial endemicity, the PCR-corrected cure rate of ACT, the type of analysis used for computing cure rate (i.e., per protocol and intention-to-treat), the molecular markers evaluated, the nature of mutations and mutations involved in treatment failure cases and the prevalence of selected mutations. All the data were independently entered by three authors in the Excel spreadsheet (Microsoft Office, Excel, USA) (AA, LPKF and SC) and discussed
4.2. Results and discussion

4.2.1. Selection process of the included studies

A total of 420 studies were retrieved from targeted electronic databases (n = 287) and by hand searching (n = 133) as presented in the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) flowchart (Fig. 1 and Fig. S3). Three hundred and ninety-nine studies were screened based on titles, abstracts and among them duplicates along with other studies from regions other than sSA and India were excluded from this systematic review. In addition, research papers such as reviews, conference papers, commentary and unrelated studies were also excluded. Finally, a total of 28 studies, 21 from sSA countries and seven from India, were shortlisted for this systematic review (Fig. 1). The list of excluded studies is presented in Supplemental material 4.

| Records identified through database searching (n = 287) | Additional records identified through hand searching, Google and Google scholar (n = 133) |
|------------------------------------------------------|--------------------------------------------------------------------------------------|
| Records after duplicates removed (n = 399)           |                                                                                      |
| Records screened (n = 399)                           |                                                                                      |
| Full-text articles assessed for eligibility (n = 49)  |                                                                                      |
| Studies included in qualitative synthesis (n = 28)   | - 21 studies in Africa                                                               |
|                                                     | - 7 studies in India                                                                 |

350 Records excluded
- Molecular epidemiology studies (n = 65)
- ACTs were not evaluated (n = 50)
- ACT efficacy studies conducted in SSA and India but with no address of molecular markers associated with drug resistance (n = 136)
- ACT efficacy studies not conducted in SSA and India (n = 65)
- Modelling study (n = 1)
- Study that analyzed two clinical efficacy studies (n = 1)
- Conference papers (n = 11)
- Review (n = 10)
- Commentary (n = 2)
- Preprint (n = 1)
- Unrelated (n = 7)

21 articles excluded
- Data not related to ACTs (n = 2)
- Studies published before 2008 (n = 9)
- Molecular epidemiology study with no ACT efficacy component (n = 4)
- Molecular epidemiology study with ACT efficacy component but no clear information on nature and prevalence of resistance mutations (n = 1)
- Molecular epidemiology study with ACT efficacy component but findings on resistance mutations were not linked to failure treatment or compared before and after treatment (n = 1)
- Molecular epidemiology study with ACT efficacy component but genotyping of resistance mutations were made at baseline or post-treatment only (n = 3)
- Resistance molecular study were analysed from archived samples of an ACT efficacy program (n = 1)

Fig. 1. PRISMA chart of the selection steps of included studies.
4.2.2. Results of bias risk assessment

As shown in Fig. S5, bias risk assessment has varied from one study to another and was mainly found for “drug allocation concealment” and “blinding treatment to patients, caregivers and outcome assessors”. Few studies were designed as either single-blinded or open-label trial (Djimdé et al., 2008; Otienoburu et al., 2016; Saha et al., 2012; Some et al., 2016; Sondo et al., 2016). It should be noted that the risk was « unclear » for several studies as the authors had not sufficiently given information on some methodological aspects including randomization, drug allocation concealment, comparability of treated groups for instance (Fig. S5). Some studies used only per protocol (PP) analysis to evaluate the ACT efficacy instead of intention-to-treat (ITT) analysis.

Table 2
Characteristics of the studies included in the systematic review.

| Author_Year | Countries (Area) | Study period | Endemicity | Study population | Antimalarial drugs evaluated | Molecular markers investigated |
|-------------|------------------|--------------|------------|------------------|-----------------------------|-------------------------------|
| **AFRICA**  |                  |              |            |                  |                             |                               |
| **AFRICA**  |                  |              |            |                  |                             |                               |
| Djimdé et al. (2008) * | Mali (Bougoula-Hameau) | 2002 to 2004 | Hyperendemic ≥6 months | AS; AS + AQ; AS + SP | Pfcr, Pfhr, Pfpps, Pfmdr1 | Fhfr, PfATPase6 |
| Hapti et al. (2009) | Nigeria (Ibadan) | 2006 to 2007 | Hyperendemic ≤10 years | AL | Pfmdr1, PfATPase6 |
| Karem et al. (2010) * | Rwanda (Mashesha and Rukara) | 2006 | Not specified 6-59 months | AS + CPG-DDS; AQ + SP | Pfhr, Pfpps, Pfmdr1 |
| Some et al. (2010) | Burkina Faso (Bobo-Dioulasso) | Not specified | Holoendemic ≥6 months | AL; AQ + SP; DHA + PPQ | Pfmdr1, Pfcr, Pfhr, Pfpps |
| Baliraine and Rosenthal (2011) | Uganda (Kampala) | 2004 to 2008 | Not specified Children (1–10 years) | AL; AS + AQ; AQ + SP | Pfmdr1 |
| Gaddala et al. (2011) * | Sudan (Asar, Darawai, and Abu Adam) | 2012 | Not specified All age | AL | Pfmdr1, PfATPase6 |
| Nsagala et al. (2011) | Tanzania (Fukayosi and Yombo) | 2007 to 2008 | Not specified 6-59 months | AL | Pfmdr1, Pfcr |
| Nsagala et al. (2011b) * | Tanzania (Ngeta and Mwanabwito) | 2007 | Not specified 3-59 months | AL | Pfmdr1, Pfcr |
| Kamugisha et al. (2012) | Tanzania (Igombwe-Mwanza) | 2010 to 2011 | Not specified 6-60 months | AL | Pfhr, Pfpps |
| Gaddala et al. (2013) | Sudan (Kassala) | 2012 | Moderate perennial All age | AS + SP | Pfcr, Pfmdr1 |
| Klaco et al. (2015) | Angola (Luanda) | 2011 to 2013 | Mesoendemic All age | AL | Pfmdr1, PfATPase6, Pfk13, Pfmdr1 |
| Wasse et al. (2015) | Somalia (Jamame, Janale and Jawhar) | 2011 | Not specified ≥6 months | AS + SP | Pfhr, Pfpps |
| Otienoburu et al. (2016) | Liberia (Nimba) | 2016 | Not specified Not specified | AL; AS + AQ | Pfcr, Pfmdr1 |
| Sondo et al. (2016) | Burkina Faso (Nanoro) | Not specified | Not specified Not specified | AL; AS + AQ | Pfcr, Pfmdr1 |
| Yeka et al. (2016) | Uganda (Apac, Mubende and Karnungu) | 2013 to 2014 | Mesoendemic 6-59 months | AL; AS + AQ | Pfcr, Pfmdr1 |
| Plicinski et al. (2017) | Angola (Benguella, Lunda Sul and Zaire) | 2015 | Mesoendemic, Hyperendemic 6-59 months | AL; AS + AQ; DHA + PPQ | Pfcr, Pfk13, Pfmdr1 |
| Wasse et al. (2017) | Somalia (Janale, Jawhar and Bosaso) | 2013 to 2015 | Moderate-to-low; Low ≥6 months | AL; AS + SP | Pfhr, Pfpps |
| Baraka et al. (2018) | The Democratic Republic of Congo (Lisungu), and Uganda (Kazo) | 2012-2014 | Not specified 6-59 months | AL; AS + AQ | Pfmdr1 |
| Davlantes et al. (2018) | Angola (Benguella, Lunda Sul and Zaire) | 2017 | Mesoendemic, Hyperendemic Not specified | AL; AS + AQ; DHA + PPQ | Pfcr, Pfmdr1, Pfk13, Pfpps |
| Kocolva et al. (2018) | Tanzania (Chamwino, Butimba, Kilhaha, and Rufiji, Kyela, Kilombero, Muheza, Nagaga and Ujiji) | 2011, 2012, 2015 | Not specified 6-60 months (2011), 6 months-10 years (2012), ≥6 months (2015) | AL | Pfcr, Pfmdr1, Pfk13, Pfpps |
| Smith et al. (2018) | Sierra Leone (Bo, Kenema and Makeni) | 2016 | Not specified 6-59 months | AL; AS + AQ; DHA + PPQ | Pfhr, Pfpps, Pfk13, Pfpps2 |
| **India**    |                  |              |            |                  |                             |                               |
| **India**    |                  |              |            |                  |                             |                               |
| Saha et al. (2012) * | West Bengal, Jalpaiguri | 2009 to 2010 | Not specified ≥6 months; >5 yrs | AL; AS + SP; AS + MQ | Pfhr, Pfpps, Pfcr, PfATPase6 |
| Mishra et al. (2012) | 25 sentinel sites | 2009 to 2010 | Not specified All age | AL; AS + MQ | Pfhr, Pfpps, Pfmdr1 |
| Ganguly et al. (2013) * | West Bengal (Purulia) | Not specified | Not specified All age | AS + SP | Pfhr, Pfpps, Pfmdr1 |
| Srivastava et al. (2013) | Ranchi, Meghalaya and Keonjhar | 2007 to 2010 | Hyperendemic All age | AS + SP | Pfhr, Pfpps |
| Mishra et al. (2014) | Arunachal Pradesh (Changlang, Miao), Tripura (Gomati, Silcharahi), Mizoram (Lunglei, Tlabung) | 2012 | Not specified All age | AS + SP | Pfhr, Pfpps |
| Mishra et al. (2017) | Balaghat and Anuppur district, Madhya Pradesh | 2012 to 2014 | Not specified ≥1 yrs | AS + SP | Pfhr, Pfpps, Pfk13 |
| Das et al. (2018) | West Bengal | 2013 to 2014 | Not specified Not specified | AS + SP | Pfk13 |

ACT: Artemisinin-based combination therapy; AL: Artemether + Lumefantrine; AS + AQ: artesunate + amodiaquine; AS + CPG-DDS: Artesunate + Chlorproguanil-Dapsone; AS + MQ: artesunate + mefloquine; AS + SP: artesunate + sulfadoxine-pyrimethamine; DHA + PPQ: dihydroartemisinin + piperaquine; GTS: Global technical strategy; Pf: Plasmodium falciparum; Pfps: PfCSP; PfCa2+: ATPase; Pfcr: Pf chloroquine resistance transporter; Pfhr: Pf dihydrofolate reductase; Pfhx: Pf cytochrome B; Pfhr: Pf dihydropteroate synthase; Pfk13: Pf Kelch gene; Pfmdr1: Pf multidrug resistance protein 1; Pfpms2: Pf plasmaspin 2.

* These studies were conducted in rural areas.
which is recommended by the JBI (Fig. S5) (Gadalla et al., 2011; Kamugisha et al., 2012; Kiaco et al., 2015).

4.2.3. Characteristics of the included studies

In Africa, the included studies were conducted in twelve countries namely Angola, Burkina Faso, Mali, Nigeria, Sierra Leone, Liberia, Sudan, Somaliland, Tanzania, Rwanda, The Democratic Republic of Congo (DRC) and Uganda and were published in years during and after 2008 (Djimdé et al., 2008; Happi et al., 2009; Karema et al., 2010; Some et al., 2010; Baliraine and Rosenthal, 2011; Gadalla et al., 2011, 2013; Ngsala et al., 2011a, 2011b; Kamugisha et al., 2012; Kiaco et al., 2015; Warsame et al., 2015, 2017; Oienoburu et al., 2016; Sondo et al., 2016; Yeka et al., 2016; Plucinski et al., 2017; Baraka et al., 2018; Davlantes et al., 2018; Kakolwa et al., 2018; Smith et al., 2018) (Table 2).

Two studies were from mesoendemic areas, three from hyperendemic areas, one each from holoendemic, mesoendemic and hyperendemic areas; though the level of endemicity was not specified in remaining studies (Davlantes et al., 2018; Djimdé et al., 2008; Happi et al., 2009; Kiaco et al., 2015; Plucinski et al., 2017; Some et al., 2010) (Table 2).

The studies evaluated the efficacy of ACT between the years 2002-2017 in children, especially those aged <5 years, and/or both children and adults. Seven studies, six in sSA and one in India, collected data before 2008, the year of first documentation of ART-resistance (Baliraine and Rosenthal, 2011; Djimdé et al., 2008; Happi et al., 2009; Karema et al., 2010; Ngsala et al., 2011a, 2011b; Srivastava et al., 2013).

ACT evaluated in the studies included in AL, AS + AQ, AS + SP, AQ + SP and DHA + PPQ (Table 3). The included Indian studies evaluated efficacy of three ACT viz AS + SP, AS + MQ and AL between 2012 and 2014 in children, adolescents and adults, among which one study was conducted in a highly endemic area, while the level of endemicity was not specified in the remaining four studies (Das et al., 2018; Ganguly et al., 2013; Mishra et al., 2012, 2014, 2017; Saha et al., 2012; Srivastava et al., 2013).

4.2.4. Efficacy of ACT

The PCR-corrected cure rates of different ACT showed large variations in studies from sSA. Based on ITT analysis, the cure rates of different ACTs were: 54.3–100% for AL, 70.7–100% for AS + AQ, 79.3–99.0% for AS + SP and 99.8–100.0% for DHA + PPQ after 28-day follow-up, 68.8–98.1% for AL, 89.1–100% for DHA + PPQ after 42-day follow-up (Table 3). ACT were highly efficacious against malaria parasites (i.e., cure rate ≥95%) in most studies while reduced efficacy (i.e., cure rate <90%) were reported in one study from Angola for AL in one of the study site (i.e. Zaire area) (Plucinski et al., 2017).

In India three ACT were evaluated in the studies included in the review namely AS + SP (six studies), AL (one study) and AS + MQ (one study). Based on ITT analysis, the efficacy of AS + SP ranged from 98.7 to 100% and from 90.6 to 97.0% after 28 days and 42 days of follow-up respectively (Table 3). ACT efficacy was >90% in all the Indian studies with only one exception for which AS + SP efficacy of 59.5–82.7% was found (Mishra et al., 2014).

ACTs were highly efficacious (i.e., cure rates >90%) at the time of data gathering despite the fact that nine studies reported cure rates <90% (8 in sSA and 1 in India) (Baliraine and Rosenthal, 2011; Gadalla et al., 2013; Karema et al., 2010; Mishra et al., 2014; Plucinski et al., 2017; Sondo et al., 2016; Warsame et al., 2015, 2017; Yeka et al., 2016) (Supplementary file 6). At least three possible hypotheses could explain this low cure rates reported in these nine studies. Firstly, AS + SP was evaluated in one third of the studies (3/9) and the high TF rates following treatment with this ACT was statistically attributable to the presence of isolates with multiple mutations in Pfhr/ Pfdfs genes (Mishra et al., 2014; Warsame et al., 2015, 2017)(Table 4). Secondly, distortions in the study design of the included studies as some of them were not designed: i) as randomized control trial (RCT), ii) open-label RCT and iii) had high bias risk for some aspects including “drug

### Table 3

| Authors, Year | ACT tested | Type of analysis | 28-day ACPR (%) | 42-day ACPR (%) |
|---------------|------------|-----------------|----------------|----------------|
| Djimdé et al. (2008) | AS + AQ PP | 99.1 | – |
|             | AS + SP PP | 100.0 | – |
| Happi et al. (2009) | AL ITT | 95.6 | – |
| Karema et al. (2010) | AS + ITT | 70.5–73.3 | – |
|               | CPG-DDS AQ + SP ITT | 38.1–87.8 | – |
| Somé et al. (2010) | AL ITT | – | 68.8 |
|               | DHA + ITT | – | 89.1–92.4 |
|               | PPQ AQ + SP ITT | – | 88.2 |
| Baliraine and Rosenthal (2011) | AL ITT | 89.1 | – |
|               | AQ + SP ITT | 79.3 | – |
| Gadalla et al. (2011) | AL PP | 95.0 | – |
| Ngsala et al. (2011a) | AL ITT | 98.2–98.8 | 95.8–98.1 |
| Ngsala et al. (2011b) | AL ITT | 95.1 | 93.0 |
| Kamugisha et al. (2012) | AL PP | 96.0 | – |
| Gadalla et al. (2013) | AS + SP PP | 90.5 | – |
| Kiaco et al. (2015) | AL PP | 91.3 | – |
| Warsame et al. (2015) | AS + SP PP | 77.8–99.0 | – |
| Oienoburu et al. (2016) | AL AQ + AQ ITT | – | – |
| Sondo et al. (2016) | AL AQ + SP PP | 84.1 | – |
| Yeka et al. (2016) | AS + AQ ITT | 70.7 | – |
| Plucinski et al. (2017) | AL PP | 86.5–96.1 | – |
| Saha et al. (2012) | AS + AQ ITT | 99.9–100.0 | – |
|               | DHA + PPQ 98.8–100.0 | – | 98.8–100.0 |
|                   | PPQ ITT 98.8–100.0 | – | 98.8–100.0 |
| Warsame et al. (2017) | AL PP | 97.6–100.0 | – |
|               | ITT 91.1–100 | – |
|                   | AS + SP PP 87.7 | – |
| Baraka et al. (2018) | AL PP | NE | NE |
| Davlantes et al. (2018) | AS + AQ PP | 95.5–96.4 | – |
|                   | DHA + PPQ 93.0–100.0 | – | 100.0 |
|                   | PPQ ITT 95.6–96.5 | – | 93.3–100.0 |
|                   | DHA + ITT 100.0 | – | 100.0 |
| Kakolwa et al. (2018) | AS + AQ PP | NE | – |
|                   | AL PP | NE | – |
|                   | DHA + PPQ 100.0 | – | NE |
| Smith et al. (2018) | AS + AQ PP | 100.0 | – |
|                   | AL PP | 100.0 | – |
|                   | DHA + PPQ 100.0 | – | 100.0 |
|                   | PPQ AS + AQ ITT | 100.0 | – |
|                   | AL ITT 100.0 | – | – |
|                   | DHA + ITT 100.0 | – | – |
|                   | PPQ AS + AQ PP | 100 | – |
| Saha et al. (2012) | AS + SP PP | 90.6 | – |
|                   | AL PP | 95.6 | – |
|                   | AS + MQ PP | 100 | – |
| Mishra et al. (2012) | AS + SP ITT | 98.8 | – |
| Ganguly et al. (2013) | AS + SP PP | 97.0 | – |
|                   | AS + ITT 100.0 | – | – |

(continued on next page)
ACT: Artemisinin-based combination therapy; ACPR: adequate clinical and parasitological response; AL: artemether + lumefantrine; AS: artesunate + amodiaquine; AS + CPG-DDS: Artesunate + Chlorproguanil-Dapsone; AS + MQ: artesunate + mefloquine; AS + SP: artesunate + sulfadoxine-pyrimethamine; DHA + PPQ: dihydroartemisinin + piperaquine; PP: per protocol; ITT: Intention-to-treat; NE: Non extractible.

4.2.5.1. Pfk13. Five studies from Africa evaluated the selective pressure of ACT on the Pfk13 propeller gene and three found wild or mutant types of this gene with absence of any selective pressure (Davlantes et al., 2018; Kakolwa et al., 2018; Kiaro et al., 2015; Plucinski et al., 2017; Smith et al., 2018). Some mutations, located in the propeller domain of the Pfk13 gene, were reported to be non-synonymous (463S, 476I, 496S, 510M, 540A, 556K, 562T, 578S, 602D and 646T) in three of these studies (Davlantes et al., 2018; Kakolwa et al., 2018; Smith et al., 2018); where the 476I mutation is validated with resistance or reduced susceptibility to ART (Table 4) (WHO, 2018). This is consistent with molecular epidemiology studies on the Pfk13 that reported the presence of other validated mutations (i.e., 561H in DRC and India) (Ménard et al., 2016; Mishra et al., 2015). The mutation 561H was found in one of the >9,000 samples from several SSA countries analyzed previously (Ménard et al., 2016).

The ART mode of action still remains elusive and controversial, though it has been shown that they cause oxidative stress by the production of reactive oxygen species (ROS) with likely impact on the multiple cellular targets (Kavishe et al., 2017). A molecule called ferriprotoporphyrin (FP) is a product of hemoglobin digestion, which thereafter polymerizes to hemozoin (malarial pigment). When ARTs are administered, these react with the FP iron (FP–Fe$^{2+}$) to form activated drugs and alongside form ROS causing attacks on several cellular targets.
Table 4 (continued)

| Authors, Year | Drug tested | Molecular markers selected or involved in ACT treatment failure | Nature and direction of selected mutations (Main findings) |
|---------------|-------------|---------------------------------------------------------------|----------------------------------------------------------|
| Warsame et al. (2015) | AS + SP | Pfmdr1, Pfcr | TF significantly associated with the presence of double mutant Pfmdr1 437G + 540E (OR = 32.4, 95%CI: 5.1–198.1), quadruple mutant Pfmdr1 51I/108N + Pfmdr1 437G/540E (OR = 5.5, 95% CI: 2.3–13.6), quintuple mutant Pfmdr1 51I/59R/108N + Pfmdr1 437G/540E (OR = 3.5, 95%CI: 1.4–8.8) |
| Otienoburu et al. (2016) | AL | Pfmdr1, Pfcr | Pfmdr1: single mutant 1246Y (+) and N86 (+); Pfcr: single mutant 76T (-) |
| | | | Pfdhfr: Haplotypes N86-Y184-1246Y (+); 865-Y184-1246Y (+); 868-Y184-121246 (-), N86-184F-121246 (+) and 865-184F-121246 (-) |
| | | | Pfcr: No evidence of selection |
| Sondo et al. (2016) | AS + AQ | Pfmdr1, Pfcr | Pfmdr1: No selection of polymorphisms; Pfcr: 76T (+) |
| | | | AL | Pfmdr1, Pfcr | Pfmdr1: No selection of polymorphisms; Pfcr: 76T (+) |
| Yeka et al. (2016) | AS + AQ | Pfmdr1, Pfcr | Pfmdr1: single mutant 86Y (+) and 1246Y (+); Pfcr: 76T (-) |
| | | | AL | Pfmdr1, Pfcr | Pfmdr1: D1246 (+); Pfcr: No evidence of selection |
| Plucinski et al. (2017) | AL | Pfk13, Pfcytb, Pfmdr1 | Pfk13 and Pfmdr1: Wild types (+) |
| | | | | Pfmdr1: Haplotypes N86-184F-12462 and N86-Y184-12462 found in most of TF |
| | | | | DHA | Pfk13, Pfcytb, Pfmdr1 | Pfk13 and Pfmdr1: Wild types (+) |
| | | | | AL | Pfmdr1, Pfcr | Pfmdr1: D1246 (+); Pfcr: No evidence of selection |
| | | | | | | Pfmdr1: Single mutant 1246Y (+) and N86 (+); Pfcr: Single mutant 76T (-) |
| Warsame et al. (2017) | AL | Pfmdr1, Pfcr | Pfmdr1: Single mutant 86Y and 1246Y; Pfcr: 76T |
| | | | | AS + SP | Pfmdr1, Pfcr | Quiniquple mutation (51I/108N + 437G/540E/581G) were found in all TF cases |
| Baraka et al. (2018) | AL | Pfmdr1 | F1184 (Borderline significant direction selection in Uganda, P-value = 0.055); N86 and D1246 (No evidence of selection) |
| Davlantes et al. (2018) | AL | Pfcr, Pfmdr1, Pfk13, Pfpm1 | All 33 TF in Al. arm carried Pfmdr1 (N86-184F-12462 and 865-184F-121246 or Pfcr (72C-V73-745-756-767) mutations associated with lumeofantin on day of failure |
| Mishra et al. (2012) | AS + Pfp, Pfmdr1, Pfk13, Pfpm1 | None | Quiniquple mutation (51I + 59R + 108N, Pfmdr1, 437G) (found in 2 TF); Quiniquple mutation (51I + 59R + 108N, Pfmdr1, 436A + 540E) (found in 2 TF) and (Pfmdr1, 59R + 108N + 164L, Pfmdr1, 436A + 540E) (found in 1 TF) |
| Mishra et al. (2012) | AS + Pfp, Pfmdr1, Pfk13, Pfpm1 | None | Single mutant 108N (found in 4 TF) |
| German et al. (2013) | AS + Pfp, Pfmdr1, Pfk13, Pfpm1 | None | Quiniquple mutation (51I + 59R + 180N, Pfmdr1, 437G) (found in 2 TF) |
and its derivatives may have slowed the emergence of resistance (Wang et al., 2012; Cheeseman et al., 2012; Tawk et al., 2010). In addition, the reduced susceptibility to CQ and amodiaquine (AQ), but increased 50% inhibitory concentration (IC50; >30 nmol/L) following treatment with artemether (Jambou et al., 2005). In contrast, a study found a lack of association between this mutation in the PfATPase6 gene and ART resistance (Cui et al., 2012).

These contradictory results imply a need for further studies addressing PfATPase6 and ART resistance. However, keeping in mind that the ART could act at different locations inside the parasite, it is likely that polymorphisms in the PfATPase6 gene or other genes modulate the susceptibility to ART and its derivatives (Cui and Su, 2009; Kavishe et al., 2017).

4.2.5.3. Pfmdr1 and Pfcrt. It was showed that orthologue genes of Pfmdr1 encodes a putative ATP-binding cassette transporter mediating multidrug resistance in Candida albicans and mammalian cell lines (Duraisingh and Cowman, 2005). The single mutations (86Y, 184Y, 1246Y) and haplotypes (N86–184F-D1246; haplotypes different from N86–184F-D1246 termed as “No N86–184F-D1246”) in Pfmdr1 were found to be selected significantly by the ACT evaluated in the included studies (Table 4). The mutation at 86Y in Pfmdr1 have been shown to be associated with increased sensitivity to structurally unrelated antimalarial drugs (MQ and ART) (Duraisingh et al., 2000). It was also reported that the 86Y mutation in this gene was positively selected by either AS + AQ or AL in three included studies (Table 4) where one study has recently shown to reduce the risk of AL failure with carriage of mutant alleles 86Y compared to wild type N86 in the Pfmdr1 gene (Baraka et al., 2018; Djimde et al., 2008; Kamugisha et al., 2012; Yeka et al., 2016). This same pattern was found for Pfmdr1 1246Y in two studies included in the review (Otionoburu et al., 2016; Yeka et al., 2016). It was shown that the mutant alleles 86Y, 184Y and 1246Y in the Pfmdr1 gene mediate the reduced susceptibility to CQ and amodiaquine (AQ), but increased in vitro sensitivity to MQ, lumefantrine and ART (Table 1) (Wurtz et al., 2014). It was reported that the haplotype N86–184F-D1246 was positively selected by AL in Nigerian children where the parasite strains carrying this haplotype were known to tolerate high concentrations of lumefantrine (Malmberg et al., 2013). Similarly, in this reported review in four included studies; three of them revealed that this haplotype was positively selected by AL, and in the remaining one study it was shown to be positively selected by AS + SP (Baliraini and Rosenthal, 2011; Gadalla et al., 2013; Happi et al., 2009; Otionoburu et al., 2016).

Pfcrt is a phosphoprotein located in the membrane of P. falciparum digestive vacuole and plays a critical role in CQ resistance by directly mediating the efflux of CQ from the parasite digestive vacuole (Bray et al., 2005). Positive selection of wild types in the Pfcrt (K76) and Pfmdr1 (N86 and Y184) genes after AL treatment (Table 4) was found in several studies (Baliraini and Rosenthal, 2011; Happi et al., 2009; Ngasala et al., 2011a, 2011b; Otionoburu et al., 2016). The reappearance of wild type alleles in parasite population is considered as a good indicator of their return to CQ sensitivity as shown by Laufer and co-workers in Malawi (Laufer et al., 2006), though with reduced sensitivity to lumefantrine and two other structurally similar drugs viz MQ and halofantrine (McCarthey and Price, 2015).

4.2.5.4. Pfδhfr and Pfδhps. Resistance to SP is conferred by polymorphisms in Pfδhfr and Pfδhps genes encoding enzymes involved in the folate-pathway essential for P. falciparum survival (Gregson and Plowe, 2005). The Pfδhfr (51I, 59R and 108N) and Pfδhps (437G) genes with single and multiple mutations were positively selected after treatment including mitochondrial membrane and transport chain, hemoglobin digesting proteases and many cytosolic targets including the cysteine-containing redox system of the parasite (Kavishe et al., 2017).

In addition, it is likely that the seemingly promiscuous nature of ART and its derivatives may have slowed the emergence of resistance (Wang et al., 2015). The authors Mbengue and colleagues established the crucial role of P. falciparum phosphatidylinositol-3-phosphate (PPI3K) in acquisition of ART resistance (Mbengue et al., 2015) and found a strong association between increased level in PPI3K and CS80Y mutation in the PfK13 gene, which revealed that K13 protein act as adapter of PI3K to control its expression levels (Kavishe et al., 2017). In addition, PPI3K may impact functions of the apicoplast and food vacuole, host remodeling and cell survival via DNA repair and transcriptional pathways which have also been implicated in ART resistance (Bhattcharjee et al., 2012; Cheeseman et al., 2012; Tawk et al., 2010). In addition, other mechanisms of actions for ART have been proposed recently (Birnbaum et al., 2020; Hou et al., 2019; Yang et al., 2019).

Among the included studies in this review, 15 studies (10 from SSA and five from India) were published before the involvement of the PfK13 gene in ART resistance was established (Ariey et al., 2014). Thus, none of these studies performed genotyping of this gene among TF cases and as a consequence it is impossible to confirm the causative role of PfK13 mutations in these studies. Though, one Indian study genotyped the PfK13 gene and the authors found a novel mutation 625R in four TF and 539T mutation (validated for ART resistance) in one TF (Das et al., 2018). Another study by the same research group, reported the presence of the same mutation (539T) in patients treated with AS + SP (Das et al., 2019). The 539T mutation is recently validated by the WHO for ART resistance (WHO, 2019). Also, the WHO and experts outlined that results of these two studies should be interpreted with caution and concluded that “it is premature to claim that ART resistance has emerged in India” (Rasmussen et al., 2019; WHO, 2019).

### Table 4 (continued)

| Authors, Year | Drug tested | Molecular markers selected or involved in ACT treatment failure | Nature and direction of selected mutations (Main findings) |
|---------------|-------------|---------------------------------------------------------------|----------------------------------------------------------|
| Das et al. (2018) | AS + SP | PfK13 | 108N (found in 32 TF) Identification of two non-synonymous mutations in PfK13 gene (579T, 657H) Identification of mutation 539T (found in 1 TF) along with a novel 625R mutation (found in 4 other TF cases) |

Molecular markers significantly lowered post-treatment; () Positive selected (i.e., the prevalence of the molecular marker significantly increased post-treatment); () Negative selected (i.e., the prevalence of the molecular marker significantly lowered post-treatment); ACT: Artemisinin-based combination therapy; AL: artemether + lumefantrine; AS: ART; AQ: artesunate + amodiaquine; AS + CPG-DDS: Artesunate + Chlorproguanil-Dapsone; AS + MQ: artesunate + mefloquine; AS + SP: artesunate + sulfadoxine-pyrimethamine; DHA + PQ: dihydroartemisinin + piperaquine; GTS: Global technical strategy; Pf: Plasmodium falciparum; Pfap6: Pf CA; -ATPase; Pfcrf: Pf chloroquine resistance transporter; Pfδhfr: Pf dihydrofolate reductase; Pfcytb: Pf cytochrome B; Pfδhps: Pf dihydropteroate synthase; PfK13: Pf kelch gene; Pfmdr1: Pf multidrug resistance protein 1; Pfpm2: Pf plasmepsin 2; TF: Treatment failure.

Mutations in the genes were found in treatment failure case. a Mutations in the genes were found in treatment failure case.

b “No N86–184F-D1246” refers to haplotypes different from N86–184F-D1246.
with AQ + SP and AS + SP, as revealed in two studies reported from Mali and Burkina Faso (Djimdé et al., 2008; Sémé et al., 2010) (Table 4) which were also reported in Sudanese and Somalian TF patients treated with AS + SP (Gadalla et al., 2013; Warsame et al., 2017). These reported mutations are known to elicit resistance to SP in malaria parasites (Naidoo and Roper, 2013; Okell et al., 2017a). This could be due to the high selective pressure exerted by the exposure to the therapeutic combination of SP which is largely used as intermittent preventive treatment in pregnancy (IPTp), seasonal malaria chemotherapy in children and ITP in infants in most African countries where malaria is endemic (Group et al., 2017; WHO, 2018a). However, reports from Indian subcontinent stated that all the Pfδfr and Pfδhps mutations were of multiple kind (double, triple, quadruple and quintuple) in TF with AS + SP as compared to the included African studies where mainly single mutations were found (Baliraine and Rosenthal, 2011; Kamugisha et al., 2012; Otienoburu et al., 2016; Sémé et al., 2010; Yeka et al., 2016). In addition, all cases of Pfδfr/Pfδhps multiple mutations from the included African studies were reported only from the West part of the continent (Djimdé et al., 2008; Otienoburu et al., 2016; Warsame et al., 2015). These two findings from our study are not in line with previous report showing a high circulation of Pfδfr/Pfδhps multiple mutations, quintuple and even sextuple mutations, in all regions of Africa (Okell et al., 2017b; Ruizendaal et al., 2017; Sridaran et al., 2010). This discrepancy can be due to the fact that we focused on studies that linked results of ACT efficacy and molecular markers associated with drug resistance. Thus, studies that only addressed the epidemiology of molecular markers associated with drug resistance got excluded from our analysis. The molecular epidemiological studies observed that Pfδfr/Pfδhps multiple mutations, especially double mutations 437G + 540E are more commonly found in East African countries than in West African countries (Sridaran et al., 2010). Higher drug pressure due to the higher utilization of AS – SP in East African countries could be responsible for this increase in the polymorphisms (WHO, 2019). Also, parasites with multiple Pfδfr and Pfδhps mutations have a higher level of in vitro resistance and have been shown to be a strong predictor for TF as compared to their single counterparts (Mombo-ngoma et al., 2011; Ndiaye et al., 2013). The presence of high prevalence of parasites with multiple Pfδfr and Pfδhps mutations found in TF cases with AS + SP from Indian studies indicates the need for an eventual switch to other ACT in the future.

4.2.4.5. PPm2 and 3. P. falciparum plasmepsin (Pfpm) 2 and 3 are located in the food vacuole and involved in the degradation of hemoglobin into yielding amino acids for protein synthesis with toxic byproduct heme (Moura et al., 2009). Treatment with PPQ leads to a depletion of ribosomes and swelling of the food vacuole with undigested hemoglobin vesicles and an accumulation of free heme in the parasite (Moura et al., 2009). Although the free heme is toxic for the parasite, inhibition of hemoglobin degradation will starve the parasite and increased Pfpm2 and 3 might help the parasite to maintain amino-acid production when hemoglobin degradation is otherwise inhibited by PPQ (Amato et al., 2017).

In our review, three studies investigated the selective pressure of DHA + PPQ on the Pfpm2 gene but found an absence of such selection and one of these studies found multiple copies of Pfpm2 but no association with DHA + PPQ failures was reported (Table 4) (Davant et al., 2018; Kakolwa et al., 2018; Smith et al., 2018). Witkowski and colleagues demonstrated a strong link between an increase in the proportion of multicopy Pfpm2 parasites and DHA + PPQ related TF rates in Cambodia (Witkowski et al., 2017). It should be noted that some strains were already ART resistant and this could explain this strong link found by these authors. Two other studies showed an increased sensitivity to PPQ in malaria strains in which Pfpm2 and 3 were inactivated (Amato et al., 2017; Mukherjee et al., 2018). In contrast, a more recent study showed conflicting results using genetically engineered laboratory malarial strains. The authors found no major changes in susceptibility to PPQ due to increased number of copies the Pfpm2 and Pfpm3 genes (Loeschanluech et al., 2019). Two other studies concluded that an increase in copy number of Pfpm2 is not required to render P. falciparum laboratory strains (Dd2) and clinical isolates resistant to PPQ (Boonyalalai et al., 2020; Ross et al., 2018).

All these above discussed data suggest that the Pfpm2 would not be the only gene involved in resistance to PPQ and DHA + PPQ failures. This is supported by findings from genetic engineering-based studies that have suggested that mutations in the P. falciparum exonuclease (Pfexo) and Pfct genes are associated with PPQ resistance and DHA + PPQ failures (Amato et al., 2017; Boonyalalai et al., 2020; Ross et al., 2018).

To date, increased copy number of Pfpm2 is considered and commonly used as a marker of PPQ resistance and DHA + PPQ failures, particularly in Southeast Asian countries as above presented (Boonyalalai et al., 2020; Huang et al., 2020; van der Pluijm et al., 2019). In contrast, studies on mutations in Pfpm2 and its link with PPQ resistance is increasingly available in Africa where mutations in this gene have been documented in high transmission areas of the continent, while in India, there is not much available information about the polymorphisms of this gene (Inoue et al., 2018). A study conducted in several sSA countries reported a low prevalence of P. falciparum isolates with reduced susceptibility to PPQ, and none of these isolates had multiple copies of Pfpm2 (Robert et al., 2018). Thus, it is necessary to study the role of Pfpm2 on PPQ resistance and DHA + PPQ failures in sSA as this ACT is being included in national treatment policies of several African countries (such as Cameroon, Senegal) (Ministere de la Sante Publique, 2019).

4.2.5. ACT and risk of drug resistance

The evidence of selection of mutant variants associated with drug resistance in the ACT suggests the importance of follow up in order to know the prevalence of sensitive strains circulating in malarious patients. In the present review it was observed that all selected and/or involved polymorphisms in TF were related to partner drug of the ART derivative; thereby stressing the importance to preserve the efficacy of partner drugs (Nsanzabana, 2019). Also, using a meta-analysis, Zhou and colleagues found a higher risk of drug resistance emergence using AS + SP compared to AL while the risk was lower in DHA + PPQ compared to AL (Zhou et al., 2016) with no difference among the following different ACT viz AL with AS + MQ or AS + AQ, and ii) AS + AQ and AS + SP (Zhou et al., 2016). Thus, a possibility to prevent the appearance of ACT resistance could be the utilization of DHA + PPQ, which has the lowest risk of ART resistance emergence, in countries where this ACT is not used. Another approach to the protection of ACT efficacy would be the cyclic utilization of other non-ACT antimalarial drugs such as CQ where decreased efficacy of ACT is reported. Some authors reported return to plasmodial sensitivity to CQ in Malawi a few years after withdrawal of this antimalarial drug thereby suggesting its reutilization, in combination with other drug as seen with ACT, as first-line treatment could be interesting approach in these areas (Lauer et al., 2006). Besides, the possibility to use ACT containing more than one partner drug (termed as “triple ACTs”), although currently controversial, could be an alternative approach as recently modelled and debated (Dini et al., 2018; Krishna, 2019; White, 2019). All these suggested steps taken together could help in achieving malaria elimination objectives of the WHO in sSA and India.

5. Limitations of the review

One of the limitations of this review could be a selection bias as only published articles written in English or French were considered for this review analysis. There is a possibility that some studies were missed even though a rigorous strategy was used to search for relevant papers. The other important limitation was lack of coverage of all African
countries where malaria is highly endemic but no studies are reported from these countries.

6. Conclusions

In conclusion, ART resistance is the main threat to malaria control in endemic countries worldwide. Firstly, in this review high efficacy of ACT in Africa and India was observed thereby, supporting the need for protection of this efficacy over space and time. Secondly, the study noted the non-synchronous mutations in the PfK13 gene and ART resistance has not emerged in SSA and India yet, despite the fact that some clinical efficacy and epidemiological studies have reported the circulation of some validated mutations (i.e., 476I, 539T and 561H) in these two highly malaria endemic areas. This review also highlights a selective action of ACT on some molecular markers associated with resistance to partner drugs and thereby supporting the fact that TF does not necessarily mean ART resistance (Krishna and Kremsner, 2013). Finally, we outline the need to pursue researches on other possible molecular markers of ART resistance such as PfATPase6 for which the role in ART resistance is still controversial. Also, the link between multiple copies of Pfpm2, PPQ resistance and DHA + PPQ failures in malaria endemic areas other than Southeast Asia area needs more research. Given the fact that DHA + PPQ pressure is increasing in SSA as this ACT is being adopted by several SSA countries, it is important to have studies on African P. falciparum isolates for PPQ resistance. It is hence crucial to implement, maintain and scale up the therapeutic efficacy surveillance systems in endemic countries in order to induce changes in treatment policies to protect the efficacy of ACT with special emphasis on partner drugs. All these strategies coupled together will thereby limit the chance of emergence and spread of ART resistance and likely achieve the elimination targets by 2030 in India and most African countries.

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Author’s contributions

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Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and in the attached supplementary files.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.
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