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Plasmodium falciparum drug resistance gene status in the Horn of Africa: A systematic review

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Antimalarial drug resistance monitoring is the key factor in malaria control policy for early detection and subsequent prevention of drug resistance spread. This review was performed to collate all available data of *P. falciparum* resistant genes in the Horn of Africa as a baseline for future appraisal of the regional malaria control policy. The search of this review was performed in January 2018 using the scientific databases Pub Med and Google Scholar. The search terms used included: *Plasmodium falciparum* AND drug resistance genes OR molecular marks AND Somalia OR Ethiopia OR Eritrea OR Djibouti. The majority of studies (9 of 18 studies, 50%) examined *pfdhfr*, *pfcrt* and *pfmdr 1* genes. Eight (44%), 4 (22%), and 2 (11%) studies analyzed *pfdhps*, *pfk 13* and *pfatp 6 gene*s, respectively. The *Pfcytbc1* associated with atovaquone resistance is the only gene with no mutation detected. High frequencies of *pfdhfr* and *pfdhps* mutations were reported with an association to treatment failure after the artemisinin-based combination therapy (ACT) - artesunate + sulfadoxine/pyrimethamine. The aminoquinoline resistance genes such as *pfmdr1,* and *pfatp 6* were only reported with low frequency. The 76T mutation of *pfcrt* ranged from 4 to 100%, while *pfmdr1* mutations at codon 86 and 184 varied depending on geographical locations. The 402V and 431K mutations of *pfatp 6* were found highly prevalent at 93 % and 58 % in Southwestern Ethiopia, respectively. The *pfk13* gene mutation at codon 622I was 2.4%, with an association to artemether-lumefantrine efficacy and delay of parasite clearance on day 3.

Key words: *Plasmodium falciparum*, Drug resistance gene, Molecular marker, Somalia, Ethiopia, Eritrea, and Djibouti.

INTRODUCTION

Malaria is an arthropod-borne disease with a major impact on the world's human population health. In 2017, 90% of malaria cases were reported from Africa, 7% from Southeast Asia and 2% from the Eastern Mediterranean

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Figure 1. Map of Horn of Africa (white area).

Region (WHO, 2017). The Horn of Africa consists of four countries, *i.e.,* Djibouti, Ethiopia, Eritrea, and Somalia (Figure 1). In 2015, about 1.9 million malaria cases were reported from Ethiopia, while 20,963 cases were reported from Somalia and 19,372 from Eritrea (WHO, 2016). Malaria exists as hypo-endemic in Djibouti, varies from hypo- to mesoendemic in Somalia, endemic or epidemic in Eritrea, while in Ethiopia, it seasonally fluctuates (WHO, 2007). The influx of refugees is exacerbating the situation of malaria in the Horn of Africa. Drug resistance is the major problem confronting malaria control in the region (Heuchert et al., 2015). *Plasmodium falciparum* has developed resistance to multiple drugs including chloroquine (CQ), sulfadoxine-pyrimethamine (SP) and recently, artemisinin (ART) resistance strains have emerged at the Thai-Cambodian border (Golassa et al., 2014; Heuchert et al., 2015). The situation has also jeopardized the use of safe, cheap and affordable drugs in poor countries where control of malaria has been inefficient (Gebru et al., 2005). The spread of antimalarial drug resistance by human migration from one country to another has to be regarded and neighboring countries need to enforce regional instead of national programs to avoid genetic drug resistance spreading among them (Bridges et al., 2009; WHO, 2007). CQ was the first antimalarial drug used in endemic areas and resistance was reported from Thailand in the late 1950's. Since then, it spread out quickly to South Asia, then to Africa in 1974, and finally reaching East Africa in 1980's (Mekonnen et al., 2014). The dispersion of CQ resistance was a paramount factor in the failure of the first malaria control and elimination efforts in the mid of 20th century (Takala-Harrison and Laufer, 2015). Ethiopia was the first country in the Horn of Africa to document CQ resistance in 1985. At that time, the resistant isolates in Ethiopia were reported from areas bordering Kenya, Somalia, and Sudan, while the central part was apparently free from resistant strains (Alene and Bennett, 1996). In 1998, Ethiopia switched to SP as the first-line treatment for uncomplicated *P. falciparum* malaria. Unfortunately, within a short period, *P. falciparum* developed resistance to SP (Lo et al., 2017; Mekonnen et al., 2014; Schunk et al., 2006). Retrospective analysis confirmed that pyrimethamine resistant strains were present in sub-Saharan Africa before implementation of SP (Alifrangis et al., 2014). In 2004, an artemisinin combination therapy (ACT) called artemether-lumefantrine (AL) became Ethiopia's first-line treatment for uncomplicated falciparum malaria (Heuchert et al., 2015; Mekonnen et al., 2014). Eritrea, which was previously using SP, shifted to artesunate-amodiaquine (AS-AQ) in 2007(Menegon et al., 2016) and Somalia adopted AS plus SP (AS-SP) as

first-line therapy in 2007 (Warsame et al., 2015). In 2016, AL became the first line drug in Somalia's national plan for uncomplicated *P. falciparum* malaria (Warsame et al., 2017).

Drug resistance monitoring is the key factor in malaria control policy for early detection and subsequent prevention of the spread of drug resistance (Takala-Harrison and Laufer, 2015). The three basic approaches used to detect antimalarial drug resistance are *in vivo* test, *in vitro* test, and analysis of molecular markers. *In vitro* test allows investigation of parasite susceptibility to antimalarial drugs in artificial culture media without the influence of host's factors. Numerous tests are available and different drugs can be simultaneously assessed with a single isolate. Nevertheless, the requirement of live parasites and highly skilled personnel are the drawbacks of this approach Bloland et al, 2003; WHO, 2010). *In vivo* test is the gold standard for guiding malaria control policy since the test accounts for the influence of parasite, host, and drug factors. However, this approach is expensive and difficult to apply in clinical settings in most endemic areas due to limited supplies and equipment, long patient's follow-up for 28 or 48 days, repeated biological samples, and requirement of experienced staff (Bloland et al., 2003). An analysis of validated molecular marker of antimalarial drug resistance is an alternative approach for detecting antimalarial drug resistance associated with previously documented mutations. These markers are useful for monitoring of antimalarial drug resistance and obtaining a picture of the situation at a particular time (Mvumbi et al., 2015). Single or multiple nucleotide mutations in diverse *P. falciparum* gene alleles are related to a wide range of antimalarial drugs resistance. The prominent genes identified up to now include *P. falciparum dihydropteroate* synthase gene (*pfdhps*), *P. falciparum* dihydrofolate reductase gene (*pfdhfr*), *P. falciparum* multidrug resistance 1 gene (*pfmdr 1*), *P. falciparum* chloroquine resistance transporter gene (*pfcrt*), *P. falciparum* Klech 13 propeller gene (*pfk13*), *P. falciparum* adenosine triphosphatase 6 (*pfatp*6), and *P. falciparum* cytochrome b1 (*pfcytb1*).

The decline in SP sensitivity is a blend of single-nucleotide polymorphisms (SNPs) in two different genes encoding enzymes involved in the synthesis of the folate cofactor which is essential for parasite growth and survival. Mutation of *the pfdhps gene* is known to be related to sulfadoxine resistance, while that of the *pfdhfr gene* is associated with pyrimethamine resistance (Hailemeskel et al., 2013; Tessema et al., 2015). Both are often investigated in combination because of their correlation to SP resistance. The resistance level depends on the number of point mutations in these genes. Thus, multiple changes in the genes are accountable for SP treatment failure in *P. falciparum* malaria (Gebru et al., 2005). Mutations related to SP treatment failure have been identified at codons 613, 540, 437, and 436 in the *pfdhps* gene and at codons 164, 108, 59, 51, and 16 in the *pfdhfr* gene (Gebru et al., 2005; Warsame et al., 2015). The presence of the *pfdhps* double mutant A437G and K540E along with the *pfdhfr* triple mutant N51I, C59R, and S108N, known as a quintuple mutant, strongly correlates with SP resistance (Warsame et al., 2017).

The mutation at K76T of the *pfcrt* gene is strongly related to CQ resistance (Golassa et al., 2014) and is also proposed to affect the susceptibility of *P. falciparum* to ART, quinine, and amodiaquine (Heuchert et al., 2015). *Pfmdr 1* mutation has been associated with resistance of *P. falciparum* to several antimalarial drugs including CQ, mefloquine, halofantrine, quinine, and ART (Eshetu et al., 2010; Heuchert et al., 2015). Mutations in both *pfcrt* and *pfmdr1* alleles are associated with CQ resistance, although mutation of the *pfcrt* gene is a stronger indicator for CQ resistance (Golassa et al., 2014; Mekonnen et al., 2014). The *pfcrt* K76T together with other *pfcrt* mutations(C72S, M74I, N75E, A220S, I356K, and R371I) is often used as markers of CQ resistance but the clinical association has not been fully validated (Wurtz et al., 2012). The *pfcrt* CVMNK wild-type can mutate either into the CVIET or CVMNT haplotype, both of which are related to the regional evolution of CQ resistance (Heuchert et al., 2015; Menegon et al., 2016). The *pfmdr1* mutant allele 86Y has been associated with a decline in CQ sensitivity in areas where the prevalence of the parasite is low to moderate (Wurtz et al., 2012). The return of CQ sensitivity was reported after long time abandonment of CQ use in several endemic countries(Mvumbi et al., 2015) and this proposes the possibility of re-introduction of CQ for treatment of *P. falciparum* malaria in the future (Golassa et al., 2014; Golassa et al., 2015). Nevertheless, the decision will require close regional monitoring because studies of CQ resistance after discontinuation of drug pressure are considerably inconsistent among parasitic populations (Golassa et al., 2015). In Ethiopia for instance, the prevalence of *P. falciparum* clinical isolates carrying CQ resistant *pfcrt* K76T varied from 16% (Mekonnen et al., 2014) to 100% (Golassa et al., 2015).

The recent emergence of *P. falciparum* ART resistance strains in Greater Mekong Sub-region (GMS) represents a challenge to the efficacy of ART and also will postpone the goal of malaria elimination by 2030 in the region (WHO, 2015). The dissemination of these strains to Africa, where the majority of deaths due to malaria occurs, will have catastrophic results (Bayih et al., 2016). Mutation of *pfatp* 6 (encodes *P. falciparum* SERCA-type ATPase 6) has initially been linked to resistance of *P. falciparum* to artemether (Heuchert et al., 2015). Currently, the *pfk13* gene mutation, firstly reported at the Thai-Cambodian border (Heuchert et al., 2015), is suggested as the accurate marker of ART resistance. The *pfcyt b1* mutation at codon 268 was associated with

delay recrudescence of parasites after atovaquone-proguanil therapy (Musset et al., 2006; Sutherland et al., 2008; Wichmann et al., 2004).

In the present review, a qualitative analysis was systematically conducted to obtain relevant data available on *P. falciparum* molecular markers of antimalarial resistance (*pfdhps*, *pfdhfr*, *pfcrt, pfmdr 1*, *pfk13, pfatp*6, and *pfcyt b1*) in the Horn of Africa as a baseline for future assessments. Knowing how malaria parasites disseminate along with monitoring the prevalence of drug-resistant markers in the high-risk endemic areas is imperative for antimalarial policymaking.

MATERIALS AND METHODS

Search strategy

The search of relevant research articles was performed in January 2018 through the two scientific databases, namely, PubMed and Google Scholar. The following medical subject heading (MeSH) terms were used: *Plasmodium falciparum* AND drug resistance genes OR molecular marks AND Somalia OR Ethiopia OR Eritrea OR Djibouti. The references of retrieved articles were searched to obtain additional relevant articles. The inclusion criteria were (a) studies related to *P. falciparum* malaria in the Horn of Africa, (b) studies that analyzed *P. falciparum* resistance molecular markers, and (c) studies presented as original research articles. The exclusion criteria were: (a) studies that only assessed the clinical efficacy of antimalarial drugs, (b) non-molecular studies, (c) studies conducted outside the Horn of Africa (d) studies without full-text articles available online, (e) review articles or case reports, or (f) articles in language other than English. Title and abstract of each article were initially screened, followed by a full-text assessment.

Data extraction

The following information was extracted from the eligible articles: first author, publication year, *P. falciparum* molecular markers (type and frequency), techniques used to detect these markers, sample size, year of sample collection, and geographic location of the study. The frequencies of mutant alleles for *pfdhps, pfdhfr, pfcrt, pfmdr1, pfk13, pfatp 6,* and *pfcyt b1* genes were then extracted. Screening, selection, and extraction of data were performed by two independent researchers. When disagreement arose at any stage of the process, a higher professional person was consulted for a final decision.

RESULTS

Study selection

A total of 81 articles were obtained from Google Scholar and PubMed databases. Six duplicate articles were removed using EndNote X7; 39 articles were excluded after screening their titles and abstracts against the inclusion criteria; and 19 articles were excluded from the review after full-text assessment due to following reasons: *in vivo* studies, studies conducted outside the Horn of Africa, studies focusing on treatment adherence, studies with repeated sample analysis, and studies with unsuitable findings. Eighteen eligible articles were therefore selected for this review (Figure 2). Seven *P. falciparum* genes were investigated in these articles: *pfdhps*, *pfdhfr*, *pfcrt, pfmdr1*, *pfk13, pfatp*6, and *pfcyt b1*. The majority of studies (9 of 18 studies, 50%) examined *pfdhfr, pfcrt*, and *pfmdr1*genes. Eight (44%), 4 (22%), and 2 (11%) studies analyzed *pfdhps*, *pfk13* and *pfatp 6 gene*, respectively (Table 1).

Antimalarial drug resistance genes

Pfdhps and pfdhfr

Of the 16 studies, 8 studies analyzed *pfdhfr* and *pfdhps* together, while 1 study analyzed only *pfdhfr* along with other genes. Mutation at codons 108N, 59R and 51I of the *pfdhfr* and at codons 540E and 437G of the *pfdhps* were reported. Eight studies reported the frequency of either *pfdhps* double mutants (K540E andA437G) or the *pfdhfr* triple mutant (S108N, C59R, and N51I) or both (Table 2).

Pfcrt

Of the 18 studies, 9 studies investigated K76T mutation, of which 3 studies, also investigated C72S mutation with 3.6% prevalence reported in 1 study. The SVMNK and CVIET at positions72-76 of the *pfcrt* gene were reported in 4 studies, while the SVMNT haplotype was reported in 2 studies. A high-frequency rate of CVIET haplotype was reported in 3 studies (Table 3).

Pfmdr1

Nine studies examined *pfmdr 1*mutation at codons 86, 184, 1034, 1042, and 1046. Of these, the N86Y was analyzed in the 9 studies and mutations were detected at codons 86, 184, and 1042. The 1042Y was the least frequently detected mutation (17% reported in one study) (Table 4).

Pfk 13

The analysis of gene mutation was performed in 4 studies; 2 studies reported mutation at codons N531I (4.0%) and R622T (2.5%), while the other 2 studies reported wild-type alleles (Table 5).

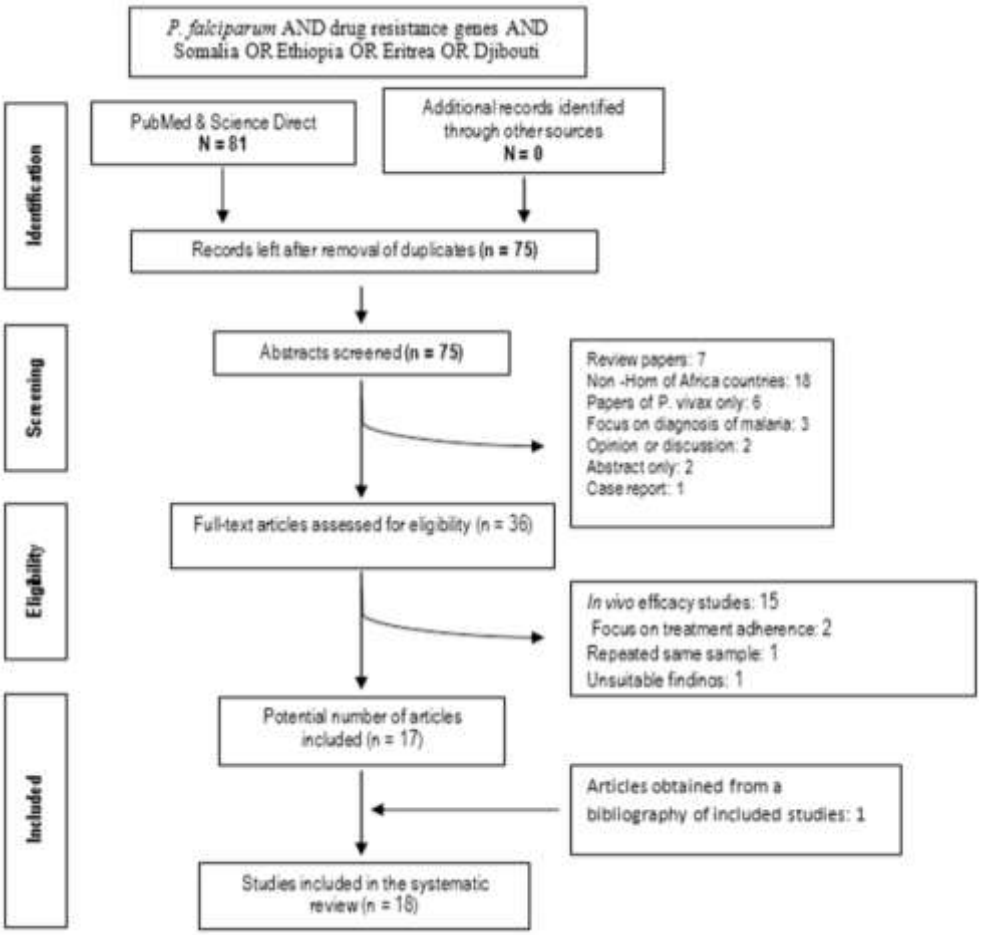

Figure 2. Flow chart showing article selection process.

Pfatp 6

Of the 18 studies, 2 investigated *Pfa*tp6 gene at different codons and a total of 12 synonymous and non-synonymous mutations (E237A, L263L, R682R, K766K, K767E, and K767R) were reported; 6 of these had not been previously depicted as resistance alleles (Table 6).

Pfcyt b1

Two (both from Ethiopia) out of 18 studies analyzed *pfcytbc1* gene mutation and none of them showed gene variation from the reference strain.

Association of molecular markers and clinical efficacy

Association of molecular markers and clinical efficacy of antimalarials was evaluated in 3 studies (2 from Somalia and 1 from Ethiopia). The clinical efficacy of AS-SP and AL were investigated with 28 days follow-up period. The PCR-corrected treatment failure was observed in 33 and 3 patients following treatment with AS-SP and AL, respectively. The cumulative efficacy of AS-SP and AL were 78 and 98%, respectively (Table 7).

The association between AS-SP clinical efficacy and the molecular markers *pfdhps* and *pfdhfr* were reported in 2015 and 2017 in Somalia (Warsame et al., 2017; Warsame et al., 2015), with failure rates of approximately 22.2 and 12.1%, respectively. For the 2015 study, the quadruple 51I/108N+437G/540E and quintuple 51I/59R/108N+437G/540E mutations of *pfdhfr+pfdhps* were shown to be associated with high risk of treatment failure with odd ratios of 5.5 and 3.5 respectively. The double 437G/540E mutation of *pfdhps* was associated with the highest risk of treatment failure (OR =22.4). On the other hand, two years later, all parasites isolated from patients with treatment failure after AS-SP carried the quintuple mutation 51I/108N + 437G/540E/581G.

The efficacy of AL was investigated in two studies in

Table 1. Characteristics of the 18 studies included in the current systematic review.

References	Study location	Study year	Sample size *	Genotyping Technique	Analyzed genes
(Heuchert et al., 2015)	Southwest Ethiopia	2013	177	Nested PCR- RFLP, Real-time PCR, Sequencing	*pfcrt, pfmdr, pfatp 6, Pfk13*
(Gebru et al., 2005)	Southwest Ethiopia	ND	124	Nested PCR, Sequencing	*pfdhps, pfdhfr*
(Golassa et al., 2014)	South-central Oromia	2012	99	Nested PCR-RFLP, Sequencing	*pfcrt-CVIET*
(Mekonnen et al., 2014)	Southeast Ethiopia	2011	195	Nested PCR, Sequencing	*pfmdr1, pfcrt*
(Lo et al., 2017)	South Ethiopia Eastern Ethiopia North Ethiopia	2014	226	Nested PCR, Real-time PCR, Sequencing	*pfcrt,pfmdr1, pfdhps, pfdhfr, pfk13*
(Schunk et al., 2006)	Southern Ethiopia	2004	69	Nested PCR-RFLP	*pfdhs,pfdhfr, pfmdr 1*
(Menegon et al., 2016)	Eritrea	2013-14	180	PCR, Sequencing	*pfcrt, pfmdr-1, pfk13*
(Warsame et al., 2015)	Somalia	2011	283	PCR, Sequencing	*pfdhfr/pfdhps*
(Warsame et al., 2017)	Somalia	2013-15	90	Nested PCR, Sequencing	*pfdhps, pfdhfr*
(Golassa et al., 2015)	East Shoa, Gambella and West Arsi of Ethiopia	2012-2014	152	PCR–RFLP, Sequencing	*pfcrt, pfmdr-1*
(Bayih et al., 2016)	Northwest Ethiopia	2013-2014	148	Nested PCR, Sequencing	*pfk 13*
(Hailemeskel et al., 2013)	Northwest Ethiopia	2005 2008	78 87	Nested PCR-Dot plot hybridization	*pfdhps,pfdhfr*
(Tessema et al., 2015)	Northwest Ethiopia	2005 2007-08	80 79	Nested PCR-Dot plot hybridization	*pfdhps,pfdhfr*
(Eshetu et al., 2010)	Southwest Ethiopia	2006	97	Nested PCR-RFLP	*pfdhfr, pfmdr1,pfatp 6,pfcytbc1,*
(Tajebe et al., 2015)	Gondar-Ethiopia	2014	133		*pfcrt, pfmdr1*
(Mula et al., 2011)	Southern Ethiopia	2007-09	66	Nested PCR-RFLP	*pfdhps,pfdhfr, pfmdr1*
(Rogier et al., 2005)	Djibouti	1998-02	139	Nested PCR, Sequencing	*pfcrt*
(Gebru et al., 2006)	Southwest Ethiopia	ND	141	PCR, Sequencing	*pfcytbc1*

*****Considering falciparum malaria samples only and samples collecting from the Horn of Africa.
ND: no data available, **PCR:** Polymerase chain reaction**, RFLP:** Restrict fragment length polymorphism.

Ethiopia and Somalia (Bayih et al., 2016; Warsame et al., 2017)**.** In the first study (Bayih et al., 2016), the association between AL clinical efficacy and the *pfk13* mutation was found. The 622I mutation was found in 2.4% of all samples with association with the delay in parasite clearance on day 3 (all mutant parasites isolated from patients who parasitemic at day 3). In the second study (Warsame et al., 2017), treatment failure rate of AL was <6%; however, no molecular marker was investigated.

DISCUSSION

A total of 18 *P. falciparum* eligible molecular studies conducted in the Horn of Africa were included in the analysis. These studies were conducted 16 years after the completion of malaria genome sequencing in 2002 (Carlton et al., 2004). Three studies particularly addressed the correlation between results of the *in vivo* assessment and molecular markers of antimalarial drug resistance (Bayih et al., 2016; Warsame et al., 2017;

Table 2. The *pfdhps/pfdhfr* mutant allele frequencies reported in the articles included in the analysis.

References	Study location	Study year	Sample size*	*Pfdhfr*mutation (%)				*pfdhps*mutation (%)		*pfdhps/ pfdhfr* Quintuple mutation (%)
				108	59	51	Triple mutation	540	437	
(Gebru et al., 2005)	Southwestern Ethiopia	ND	124	100	54.0	100	54.0	100	100	54.0
(Lo et al., 2017)	North Ethiopia	2014	65	52.3	23.2	52.3	ND	ND	15.6	ND
	South Ethiopia		62	79.0	66.0	77.4	ND	ND	15.0	ND
	East Ethiopia		72	100	59.7	100	ND	ND	14.0	ND
(Schunk et al., 2006)	Southern Ethiopia	2004	69	100	90.0	97.0	87.0	97.0	97.0	86.0
(Warsame et al., 2015)	Jamame, Somalia	2011	88	100	52.3	79.6	31.8	59.3	61.6	24.4
	Janale, Somalia		79	100	35.4	86.1	27.9	7.6	7.6	5.1
	Jowhar, Somalia		102	100	54.9	94.1	49.0	41.2	41.2	15.7
(Warsame et al., 2017)	Bosaso, Somalia	2013-2015	90	100	22.0	100	22.2	ND	ND	11.1
(Hailemeskel et al., 2013)	Northwest Ethiopia	2005	61	98.4	80.3	98.4	78.6	75.4	95.1	60.6
		2008	78	98.7	56.4	98.7	56.4	64.1	97.4	37.2
(Tessema et al., 2015)	Northwestern Ethiopia	2005	63	92.1	82.5	61.9	51.0	80.0	75.4	40.7
		2007-2008	63	74.6	55.6	25.4	16.0	63.1	67.7	13.6
(Eshetu et al., 2010)	Southwest	2006	97	100	87.6	98.8	83.3	ND	ND	_ND
(Mula et al., 2011)	Southern Ethiopia	2007-09	66	100	90.8	97.4	90.8	68.4	92.1	82.9

ND: no data available, *pfdhfr: P.falciparum* dihydrofolate reductase, *pfdhps*: *P. falciparum* dihydropteroate synthase.

Warsame et al., 2015). The development and spread of antimalarial drug resistance is attributed to several factors including inappropriate dosing, poor treatment practice, substandard or counterfeit drugs, the use of ART monotherapy, and the ability of the parasite to modify its genome at any time (Bloland et al., 2003). Even though the usual treatment for malaria has followed the World Health Organization (WHO)'s recommendation Bloland et al., 2003; WHO, 2010), antimalarial drug resistance has continuously been emerging. In this systematic review, the frequencies of *P. falciparum* gene polymorphisms linked with different types of antimalarial drug resistance were analyzed. *Pfcyt bc1* associated with atovaquone resistance is the only gene with no mutation detected since the drug has never been used in this part of Africa.

The high level of *pfdhps/pfdhfr* mutations shown in the majority of the extracted data is likely caused by the wide use of SP prior to AL introduction in the region, or the concurrent use of SP in some countries in the region for intermittent prophylactic treatment for pregnancy (IPTp) and children as recommended by WHO WHO, 2010). In a study conducted in the northern zone of Somalia, the prevalence of the *pfdhfr* mutant alleles 59R, 51I, and 108N were 100, 22 and 100%, respectively. The *pfdhfr* triple mutations (51I,59R, and108N)associated with pyrimethamine

Table 3. The *pfcrt* mutant allele and haplotype frequencies reported in the articles included in the analysis.

References	Study location	Study year	Sample size*	K76 T mutation (%)	Haplotype(*pfcrt*72-76)		
					CVMNK Wild-type (%)	CVMNT mutation (%)	CVIET mutation (%)
(Heuchert et al., 2015)	Southwest Ethiopia	2013	159	4.4	50	0	50
(Golassa et al., 2014)	South-central Oromia	2012	99	100	0	ND_	100
(Mekonnen et al., 2014)	Southeast Ethiopia	2011	195	15.9	95.9	4.1	ND
(Lo et al., 2017)	North Ethiopia	2014	65	57.0	ND	ND	ND
	South Ethiopia		62	54.8	ND	ND	ND
	East Ethiopia		72	62.5	ND	ND	ND
(Schunk et al., 2006)	Southern Ethiopia	2004	69	100	ND	ND	ND
(Menegon et al., 2016)	Eritrea	2013-14	180	84.5	ND	ND	84.5
(Golassa et al., 2015)	East Shoa-Ethiopia	2012-14	31	100	ND	ND	ND
	Gambella-Ethiopia		22	72.7	ND	ND	ND
	West Arsi-Ethiopia		99	100	ND	ND	ND
(Tajebe et al., 2015)	Gondar-Ethiopia	2014	133	54.9	ND	ND	ND
(Rogier et al., 2005)	Djibouti	1988-02	139	93	ND	ND	ND

ND: no data available

resistance was 22% of the isolates. Likewise, the prevalence of *pfdhps* double mutation (437G and 540E) was 24%. The PCR-corrected AS-SP treatment failure rate reported in the study was 12% (Warsame et al., 2017). The findings of this study support previous work conducted in Jamame in the Southern zone of Somalia for the relatively high failure rate (22%) after AS-SP treatment (Warsame et al., 2015). This most likely occurred due to SP resistance since both studies showed high prevalence rates of mutation rate in the *pfdhps/pfdhfr* genes (Warsame et al., 2017; Warsame et al., 2015). In the Jamame study, the odds ratio (OR) of treatment failure within 28-days follow-up period was increased among patients harboring the *pfdhps/pfdhfr* quintuple mutant (OR 3.5,95% CI=1.4-8.8) and those carrying *pfdhps* double mutant (OR 22,95% CI:5.1-98.1) (Warsame et al., 2015). A study from Ethiopia reported mutations at codons R59, I51, and N108 in the *pfdhfr* gene at the frequencies of 100, 91 and 98%, respectively. The frequency of triple mutation (51I, 59R, and 108N) of this gene was 22%, while that of the *pfdhps* mutations at codons 437G, 540E, and both (437G and 540E) was 68, 92 and 91%, respectively (Mula et al., 2011). The observation of high prevalence rate corroborates with findings from Congo and Ghana. This indicates that SP resistance had not yet been declined (Marks et al., 2005; Ndounga et al., 2007). In the above-cited studies from the northern zone of Somalia and Ethiopia, the quintuple (double *pfdhps* plus triple *pfdhfr*) mutation which is used as a marker of SP resistance was 11 and 83% respectively. The SP failure due to *pfdhfr* triple mutation alone has been suggested in other reports from Kenya (Nzila et al., 2000) and Cameroon (Basco et al., 2000). It is worth noting that two studies from northwest Ethiopia investigated *pfdhfr/pfdhps* genes at two different time points (2005/2008) recording a marked reduction in *pfdhfr* triple mutation and *pfdhps/pfdhfr* quintuple mutation in field isolates. Nevertheless, this is not sufficient to conclude the return of SP sensitivity since a high rate of mutation was found (Hailemeskel et al., 2013; Tessema et al., 2015).

Eight out of the nine studies detected a high mutation at the core codon 76T of the *pfcrt* gene, the CQ resistance molecular marker (Mekonnen et al., 2014). The WHO recommends withdrawal of a drug if the prevalence of resistance exceeds 10% Bloland et al., 2003). The 76T mutant often occurred concurrently with other mutants, though their role has not been fully defined (Golassa et al., 2015). In Ethiopia for instance, the frequency of 76T mutation was 100% in a study conducted in South-central Oromia in 2012 (Golassa et al., 2014), while a year later, the frequency of 4.4% was reported in a similar study conducted in Southwest Ethiopia (Heuchert et al., 2015). The recovery of CQ susceptibility is a controversial issue in this country since CQ is still used for management of *P. vivax* malaria in areas with CQ resistant *P. falciparum*, resulting in a persistent selective pressure of *pfcrt* 76T (Golassa et al., 2015). Another reason is the availability of CQ from the drug stores and self-medication without the prescription

Table 4. The *pfmdr1* mutant allele and haplotype frequencies reported in the articles included in the analysis.

References	Study location	Study year	Sample size	Gene Locus						Gene copy number	
				86		184		1246			
				Mutation (%)	Wild-type (%)	Mutation (%)	Wild-type (%)	Mutation (%)	Wild-type (%)	1.5-2.5	>2.5
(Heuchert et al., 2015)	Southwest Ethiopia	2013	163	1.2	98.8	100	0	ND	ND	ND	ND
(Mekonnen et al., 2014)	Southeast Ethiopia	2011	195	14.9	85.1	5.1	94.9	0	100	ND	ND
(Lo et al., 2017)	North Ethiopia	2014	65	5.7	94.3	86	14	ND	ND	ND	ND
	South Ethiopia		62	5	95	85.5	13.5	ND	ND	ND	ND
	East Ethiopia		72	11.5	88.5	100	0	ND	ND	ND	ND
(Schunk et al., 2006)	Southern Ethiopia	2004	69	65	19	_ND	ND	ND	ND	ND	ND
(Menegon et al., 2016)	Eritrea	2013-2014	160	11.2	88.8	85.5	14.5	ND	ND	ND	ND
(Golassa et al., 2015)	Adama this.	2012-2014	30	23.3	76.7	__ ND	ND	ND	ND	ND	ND
	Gambella-Ethiopia		23	26.1	73.9	ND	ND	ND	ND	ND	ND
	West Arsi-Ethiopia		50	2	98	ND	ND	ND	ND	ND	ND
(Eshetu et al., 2010)	Southwest Ethiopia	2006	97	84.5	_ND	ND	ND	ND	ND	ND	ND
(Tajebe et al., 2015)	Gondar-Ethiopia	2014	133	45.9	54.1	ND	ND	ND	ND	25.6	28.6
(Mula et al., 2011)	Southern Ethiopia	2007-09	66	32.9	_ND	ND	ND	17.1	ND	ND	ND

ND: no data available.

due to poor management of drugs. However, a study from Kenya reported partial re-emergence of CQ sensitive strains after removal of selective pressure for this drug; thus this appears to be a country-specific drug change policy (Mwai et al., 2009). Studies conducted in Ethiopia and Eritrea identified the CVIET haplotype at the frequencies of 50 and 85%, respectively (Heuchert et al.,2015; Menegon et al., 2016), while the frequency reported in another study in Ethiopia was 0% (Mekonnen et al., 2014). Two Ethiopian studies aiming at an investigation of the SVMNT haplotype reported low mutant frequency (4 and 0%) (Heuchert et al., 2015; Mekonnen et al., 2014). The CVIET and SVMNT haplotypes are related to geographic origin of CQ tolerance (Mekonnen et al., 2014), the first haplotype predominates in Africa and Southeast Asia, whereas the other is more prevalent in some South American countries (Mehlotra et al., 2001). Concerning *pfmdr1* polymorphisms, disparate codons (86Y, 184F, 1242D, and 1246Y) with different mutation frequencies were noted in isolates from Eritrea and Ethiopia. The majority of studies showed mutation at codons 86Y and 184F. The 86Y is associated with a decline in the CQ sensitivity. Two studies in Southwest Ethiopia reported the decline in the prevalence of *pfmdr1* 86Yfrom 85% in 2006 to 1.2% in 2013 (Eshetu et al., 2010; Heuchert et al., 2015), while another study in Gondar Ethiopia reported the prevalence of 46% in 2014 (Tajebe et al., 2015). The frequency variation in these studies is likely reflecting the discrepancy of drug pressure in different geographical areas. The prevalence of the *pfcrt* 76T point mutation (55%) and high copy number variability (CNV) at codon *pfmdr1* 86Y from Gondar study, could provoke resistance for the new drug AL and delay the re-introduction of CQ in this location. In this study, high frequency of *P. falciparum* multidrug resistance copy number variants (54.2%) with 25.6% of isolates carried

Table 5. The *pfk13* mutant allele and haplotype frequencies reported in the articles included in the analysis.

References	Study location	Study year	Samples successfully typed at *pfk13*	Mutations found		
				N531I	R622T	Others
(Heuchert et al., 2015)	Southwest Ethiopia	2013	25	4.0%	ND	0.0%
(Lo et al., 2017)	Ethiopia	2014	199	No mutation found from samples analyzed		
(Menegon et al., 2016)	Eritrea	2013-2014	160			
(Bayih et al., 2016)	Northwest Ethiopia	2013-2014	125	ND	2.5%	0.0%

ND: no data available.

Table 6. The *pfatp 6* mutant allele and haplotype frequencies reported in the articles included in the analysis.

References	Study location	Study year	*Pfatp 6* gene, Codon, (N, %)					
			L402V Mutant (n/N, %)	E431K Mutant (n/N, %)	E237A Mutant (n/N, %)	L263L Mutant (n/N,%)	A623E/R682R/N683E/N683K (% for each)	K766K/K767E/K767R/S769N (% for each)
(Heuchert et al., 2015)	Southwest Ethiopia	2013	0/32 (0)	9/48 (18.8)	ND	ND	ND	ND
(Eshetu et al., 2010)	Southwest Ethiopia	2006	14/15 (93.3)	7/12 (58.3)	1/7 (14.3)	1/7 (14.3)	1/23 (4.4)	1/27 (3.7)

ND: no data available.

Table 7. Clinical efficacy of antimalarial drugs used in the Horn of Africa reported in the articles included in the analysis.

References	Study location	Study year	Sample size	Age range (yr)	Treatment regimen	Follow up (days)	Treatment failure rate on day 28 (%)
(Warsame et al., 2015)	Jamame, Somalia	2011	89	0.5-60	AS-SP	28	22.2
	Janale, Somalia		92				4.4
	Jowhar, Somalia		102				1.0
(Warsame et al., 2017)	Bosaso, Somalia	2013-2014	90	1-55	AS-SP	28	12.1
			90	1-60	AL		2.3
	Janale, Somalia	2013-2014	94	1-58	AL	28	0.0
	Jowhar, Somalia		100	2-36			1.0
(Bayih et al., 2016)	Northwest Ethiopia	2013-2014	148	1-69	AL	28	0.0

AL: artemether-lumefantrine, SP: sulphadoxine-pyrimethamine, AS: artesunate.

multi-copies (1.5- 2.5 copies) and 28.6% (> 2.5 copies) were found. This is likely to be a risk factor of resistance development for the artemether (A) partner (lumefantrine), since AL is Ethiopia's first-line treatment for falciparum malaria but the prevalence rate of multidrug resistance CNVs was not statistically significant (P>0.05) (Tajebe et al., 2015). *In vitro* study at the Thai-Cambodian border showed that parasites with higher copy number were

remarkably decreased susceptibility to lumefantrine, mefloquine, and artesunate (Lim et al., 2009).

Artemisinins are potent and rapid-acting compounds against multidrug-resistant *P. falciparum* strains. Eckstein-Ludwig *et al* suggested that artemisinins selectively inhibit *Pfatpase6*, the only SERCA-type Ca^{2+}-ATPase in the *P. falciparum* genome (Ariey et al., 2014). Two studies have targeted this gene; the sequencing of field isolates reported by Jimma *et al* (Eshetu et al., 2010) showed a variety of previously identified (L402V, E431K, A623E, N683, N683K, and S769N) and novel (E237A, L263L, R682R, K766K, K767E, and K767R) mutations in the *pfatp6* gene. Most of these mutations are globally dispersed as reports from South Africa and Asia indicate, but they were not correlated with ART resistance except for the S769N mutant being associated with *in vitro* resistance. An extremely low prevalence rate (3.7%) of S769N mutation was reported (Eshetu et al., 2010). The prevalence of the *pfatp 6* E431K mutation was declined from 58.3% in 2006 (Eshetu et al., 2010) to 18.8% in 2013 (Heuchert et al., 2015)**.** This did not occur under ART selective pressure because the drug was still in use and in addition, E431K was not detected along with A623E mutations which have been associated with a reduction of ART sensitivity. The recent emergence of ART-resistant strains in the Southeast Asian region has led many African countries to adopt ACT as the first-line treatment for uncomplicated *P. falciparum* malaria to become more vigilant since GMS was the previous spreading origin of CQ and SP resistance parasites. However, four studies conducted in Ethiopia showed no mutation at codons580Y, 543T, and 493H in the *pfk13* propeller domain which was previously correlated with artemisinin resistance in Southeast Asia (Ménard et al., 2016). These studies are in accordance with reports from Sub-Saharan Africa (Taylor et al., 2015). Only 1(4%) isolate was found harboring a new mutation at codon N531I in one study (Heuchert et al., 2015)**,** while in another study, 3 (2.5%) isolates were identified as having a novel mutation at R622T. Only one out of the three patients was found malaria positive on day-3 by microscopy with no information on *in vitro* parasite sensitivity. Interestingly, the day-3 positive patients cleared the parasite before day-28 (Bayih et al., 2016). This is unlikely to be resistance strains due to the lack of strong relationship between K13-propeller domain and delayed parasitic clearance after ART treatment as detected in Southeast Asia (Ariey et al., 2014).

Conclusions

This systematic review presents a picture of the geographical distributions of *P. falciparum* drug resistance genes during 1998-2014. The distribution pattern could be changed over time and it will be crucial to maintaining tracking through systematic sampling around lineage distribution boundaries. In this regard, a complete picture of *P. falciparum* resistance allele distribution can be generated. This record will enhance our knowledge of the real challenges on spreading of *P. falciparum* molecular markers at the extremes of the geographical distributions outlined here. Limitation of the current review includes the lack of standardized reporting layout of the data extracted from the published articles. Information on the prevalence of some point mutations was not available. Some articles included in the analysis still require a certain level of interpretation due to variations in methodological and reporting format. Furthermore, molecular studies with high prevalence mutant alleles were more likely to be published than those with wild-type alleles.

ABBREVIATIONS

AL, artemether–lumefantrine, **SP,** salphadoxine-pyramethamine, **AS,** artesunate, **AQ,** amo-diaquine, **CQ,** chloroquine, **ART,** artemisinin, **ACT,** artemisinin based-combination therapy, **SNPs,** single nucleotide polymorphisms, **GMS,** greater Mekong sub-region, **WHO,** world health organization, ***pfdhps*****,** *P. falciparum dihydropteroate* synthase gene, ***pfdhfr*****,** *P. falciparum* dihydrofolate reductase gene, ***pfmdr1*****,** *P. falciparum* multidrug resistance1 gene, *pfcrt*: *P. falciparum* chloroquine resistance transporter gene, ***pfk13*****,** Klech 13 propeller gene, ***PfATPase 6*****,***P. falciparum* adenosine triphosphatase 6, **CNV,** copy number variability, **ND,** no data available, **MeSH,** medical subject headline, **PCR,** polymerase chain reaction, **RFLP,** restriction fragment length polymorphism.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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