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Malaria resistance: a systematic review of *Plasmodium falciparum resistance* to antimalarials drugs in Democratic Republic of Congo since 1983 to 2017.

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ABSTRACT

Malaria remains a major public health problem in the DRC and the leading cause of consultation, hospitalization and death. But these efforts are likely to be wiped out by increasing the emergence and spread of resistance to antimalarials which pose a serious public health problem. Since 1983, when chloroquine-resistant strains were discovered up to the present day, what are the different studies on the resistance of *Plasmodium falciparum* to antimalarials in the Democratic Republic of Congo?

The search for this review was conducted in March 2017 using the electronic databases Pub Med Yahoo and Google. The search terms "malaria", "democratic republic of the Congo", "resistance", "antimalarial", "effectiveness" were used.

Of the 33 publications included, we found 11 articles on the therapeutic efficacy test only, 4 articles on the therapeutic efficacy test and culture *in vitro* association, 16 articles on the molecular markers only and 2 on the molecular markers and the therapeutic efficacy test. Two anti-malarial molecules (chloroquine, amodiaquine), six combinations of antimalarial drugs (sulfadoxine-pyrimethamine, artesunate-amodiaquine, arthemeter-lumefantrine, pyronadine-artesunate, dihydroartemisine-piperaquine and amodiaquine-sulfadoxine-pyrimethami ne and five molecular markers (pfcrt, Dhfr, k13) were evaluated in these publications. However, it is difficult to generalize the results of these studies throughout the Democratic Republic of Congo because these results are different from one site to another.

After thirty-four years of the resistance of *Plasmodium falciparum* to antimalarials drugs in the Democratic Republic of Congo, there are only thirty-three published papers. It is important to increase the number of studies and publications on resistance in order to have a clear situation for the whole country.

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BACKGROUND

Malaria remains a major public health problem in the DRC and the leading cause of consultation, hospitalization and death. DRC is still paying a heavy toll due to malaria. Every hour, somewhere in the country, "at least 3 families are bereaved because of malaria and more than two children are killed because of this disease" [PNLP, 2015]. The annual incidence rate has fluctuated between 2010 and 2014 between 13,591 and 13,155 per 100,000 populations, with epidemic outbreaks noted in 2013. The proportional morbidity rate also declined slightly from 2010-2014, from 36% to 32% [PNLP, 2015].

However, in the DRC, remarkable progress has been made in the reduction of malaria morbidity and infant mortality. Proportionate morbidity in children under 5 years of age decreased from 41% in 2010 to 37% in 2014, a 10% reduction. Infant mortality declined by 37%, from 92 ‰ in 2010 to 58 ‰ in 2014, 33% related to malaria prevention [PNLP, 2015].

But these efforts are likely to be wiped out by increasing the emergence and spread of resistance to antimalarials which pose a serious public health problem .Chloroquine, the molecule most used , but unfortunately developed resistance which reached DRC in the 1983 [DELACOLLETTE et al.,1983]. Plasmodium falciparum is now resistant to all antimalarials used even to the latest marketed artemisinin-based combinations. Prophylactic or therapeutic failures lead to the re-emergence of malaria with increased transmission, morbidity and mortality [PRADINES et al., 2010].

In 2015, MVUMBI et al. published a review article on the molecular markers of resistance of Plamodium falciparum to antimalarials from 1999 to 2014 (15 years). It seems appropriate to analyze this

resistance from 1983 to 2017 in order to update the data.

METHODS

This research brought together papers and reports on the resistance of Plasmodium falciparum to antimalarials in the DRC. The search for this review was conducted in March 2017 by using the electronic databases Pub Med, Yahoo and Google scholar. The search terms "malaria", "democratic republic of the Congo", "resistance", "antimalarial", "effectiveness" were used. Unpublished studies were identified through personal contacts and by manually searching the reference lists of studies identified by the above-mentioned methods, contacting individual researchers working in the field, and examining WHO records. For all studies identified, the corresponding author was contacted and asked to provide his last results. The selection criteria were as follows: a) published article on resistance in the DRC (tests for the effectiveness of therapeutic malaria in the DRC, molecular markers of malaria resistance in the DRC, ex-vivo sensitivity of Plasmodium falciparum to antimalarials in the DRC, b) Reports on chemosensitivity studies in the DRC. A total of 31 papers and two activity reports were identified. The articles were classified according to the year of collection, the year of publication, the sample size, the type of study (tests for the efficacy of malaria in the DRC, molecular markers for malaria resistance In DRC, ex-vivo sensitivity of Plasmodium falciparum to antimalarials in the DRC).

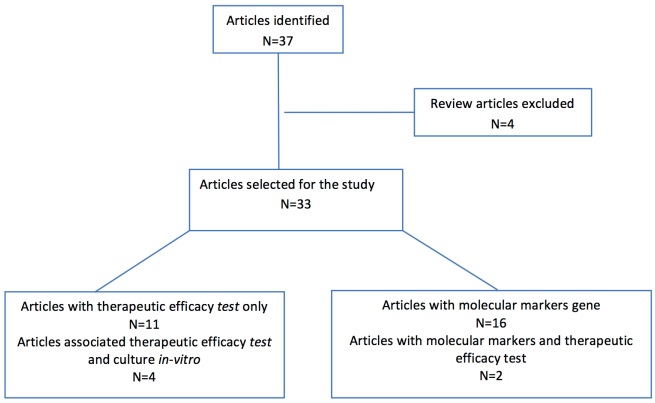

Figure 1| Research strategy: Three-three retained after the application of the inclusion criteria

RESULTS

A total of 35 articles and two activities reports were identified, of which 4 articles were review articles. In the end, we analyzed 33 articles (31 articles and two reports). Of the 33 articles selected, 11 articles dealt with studies on therapeutic efficacy in vivo test, 4 articles associated the studies therapeutic efficacy test in vivo and culture in vivo, 16 articles on marker studies of the resistance and 2 articles associated molecular markers and in vivo test. Therapeutic efficacy tests of the following molecules or combinations were carried out in the DRC: Chloroquine (6 studies), artesunate-amodiaquine (4 studies), artesunate-sulfadoxine-pyrimethamin-Pyrimethamine (2 studies),arthemeter-lumefantrine (2 studies). The genes of resistance to the following antimalarial molecules were studied: Pfcrt (Plasmodium falciparum chloroquine resistance transport) and Pfmdr (plasmodium falciparum multidrug resistant) for resistance to chloroquine and amodiaquine,Dhfr gene (dihydrofolate reductase) for resistance to pyrimethamine,Dhps gene (Dihydropteroate Synthetase) for resistance to sulfadoxine,K13 gene (propeller kelch 13) for resistance to artemisinin..

DISCUSSION

Over the past thirty-four years, we have listed thirty-three articles according to our selection criteria for malaria parasite resistance to malaria in the Democratic Republic of Congo. Indeed, chloroquine-resistant strains of Plasmodium falciparum have been reported since 1977 in eastern Africa, especially in Kenya and Tanzania, whereas African strains were previously considered as sensitive. However, DELACOLLETTE et al. [1983] reported the presence of chloroquine-resistant strains in eastern Democratic Republic of Congo, and during the same year, NGUYEN et al. conducted an investigation in Kinshasa and Mbujimayi, and find a good susceptibility of Plasmodium falciparum to chloroquine. NGIMBI et al. [1985] conducted an in vivo study in Kinsuka, a suburb of Kinshasa, in 101 malaria patients treated with chloroquine (Nivaquine) at a dose of 25 mg / kg body weight . The resorption of chloroquine was effective in all these patients. The 5 cases of probable resistance were all observed in the group of children under 4 years of age (15%). And the in vitro study confirmed 1 case, so one could conclude that there are chloroquine resistant strains of Plasmodium falciparum in Kinshasa. The resistance observed was of level RI, characterized by a disappearance of the parasitemia on day 4 and a recrudescence on day 7 [NGIMBI et al., 1985].

Several studies confirmed resistance to chloroquine across different sites in the country. In light of this increased resistance of CQ, seven studies conducted across the country from May 2000 to November 2001 showed that resistance varied from 29 to 80% for chloroquine and 0-18% for sulfadoxine-pyrimethamine (SP). From 2002 to 2004, six studies were conducted to monitor the therapeutic efficacy of SP and to evaluate AQ-SP, SP-AS and AQ-AS targeted as alternatives. This monitoring of the therapeutic efficacy of SP revealed rates of treatment failures varying between 2 and 61 % [PNLP, 2007].

Table I| Summary of *in vivo* test

Authors	year of publication	Sample size	Age	Drugs
Delacollette *et al.*	1983	46	6months-6years	CQ
Nguyen *et al.*	1985	109	>5	CQ
Ngimbi *et al.*	1985	101	6-59months	CQ
Kazadi *et al.*	2003	743	6-59 months	CQ, SP
Mbanzulu *et al.*	1988	200	>5	CQ
Paluku *et al.*	1988	339	under 5years	CQ
Onyamboko *et al.*	2014	684	3-59 months	As-AQ,A-L,DHA-PPQ
Bonnet *et al.*	2009	394	6-59months	AS-AQ,SP,AQ,AS-SP
Espie *et al.*	2012	301	6-59months	AS-AQ, A-L
Tshefu *et al.*	2010	1272	6-59months	Pyr-AS, A-L
Muhindo *et al.*	2016	865	6-59months	AS-AQ
Swarthout *et al.*	2006	180	6-59months	AS-AQ,AS-SP
Alker *et al.*	2008	249	6-59months	AS-SP,AQ-SP,SP

CQ(chloroquine),AS(artesunate),AQ(amodiaquine),SP(sulfadoxine-pyriméthamine),A-L(arthemeter-luméfantrine)

Tableau 2| Summary of *ex- vivo*

Summary of ex- vivo	test drugs	in vitro resistance (%)
MBANZULU *et al.*	CQ	0
NGIMBI *et al.*	CQ	5,5
NGUYEN *et al.*	CQ	0
DELACOLLETTE *et al.*	CQ	0

QN :quinine,CQ :Chloroquine

Table 3| Summary of molecular resistance studies

Authors	Year of publication	Samples size	Age	Molecular markers
Antonia *et al.*	2014	180	>15years	pfcrt
Taylor *et al.*	2014	151	>15years	dhps
Taylor *et al.*	2015	151	>15years	K13
Juliao *et al.*	2013	12	19-55years	pfcrt
Kamau *et al.*	2014	82	> 6years	K13
Mvumbi *et al.*	2013	145	6-59years	pfcrt
Patel *et al.*	2014	74	-	pfcrt
Pearce *et al.*	2009	177	-	dhfr
Mobula *et al.*	2009	142	1-10 years	10) pfcrt, pfmdr1, dhfr,dhps
Severin *et al.*	2005	32	-	pfcrt
Ashley *et al.*	2014	120	-	K13
Wilson *et al.*	2005	56	6-59 months	pfcrt
Cohuet *et al.*	2006	458	-	dhps+dhfr
Andriansoanirina *et al.*	2010	3	-	pfcrt
Swarthout *et al.*	2006	180	6-59months	Dhps+Dhfr
Alker *et al.*	2008	249	6-59months	dhfr+dhps
Baraka *et al.*	2017	264	-	dhps
Mvumbi *et al.*	-	580	-	pfcrt,pfmdr1,k3

Table 4| Distribution of resistance according to drugs used for therapeutic efficacy test

Authors	Drugs (%)								
	CQ	SP	AQ	ASAQ	AL	AQSP	ASSP	Pyr-AS	DHA-PPQ
Delacollette *et al.*	13	-	-	-	-	-	-	-	-
Nguyen *et al.*	-	-	-	-	-	-	-	-	-
Ngimbi *et al.*	5	-	-	-	-	-	-	-	-
Kazadi *et al.*	45,4	7,5	-	-	-	-	-	-	-
Mbanzulu *et al.*	2,3	-	-	-	-	-	-	-	-
Paluku *et al.*	28	-	-	-	-	-	-	-	-
Onyamboko *et al.*	-	-	-	6,6	7,3	-	-	-	5,7
Bonnet *et al .*	-	35,9 vs 19,6	18,3	15,1	-	-	24,6	-	-
Espie *et al.*	-	-	-	1,7	0,9	-	-	-	-
Tshefu *et al.*	-	-	-	-	0,8	-	-	0,5	-
Muhindo *et al.*	-	-	-	7,2	-	-	-	-	-
Swarthout *et al.*	-	-	-	6,8	-	-	19,7	-	-
Alker *et al.*	-	-	-	-	32	21	-	-	-
PNLP(2002-2006)	80	61	-	2	-	32	20	-	-

Table 5| Distribution of mutations and haplotypes encountered on the Pfcrt gene

Authors	Years of publication	Mutant pfcrt 76 T(%)	Haplotypes found
Antonia *et al.*	2014	55,4	CVMNK,CVIET, CVMNT,CVMDK
Juliao *et al.*	2013	8	CVMNK,CVIET
Mvumbi *et al.*	2013	73,2	CVMNK,CVIET
Mobula *et al.*	2009	83,8	-
Severin *et al.*	2005	100	CVIET,CVMNT, SVIET
Wilson *et al.*	2005	93	-
Andriansoanirina *et al.*	2010	100	-
Mvumbi *et al.*	2017	65,7	CVIET
Patel *et al.*	2014	8	CVIET

Table 6| Distribution of mutations and haplotypes encountered on the Dhps and Dhfr genes

Authors	Years of publication		Haplotypes Dhps					Haplotypes Dhfr			
		Codon	436	437	540	581	613	51	59	108	164
		Wild haplotypes	S	A	K	A	A	N	C	S	I
		Mutants haplotypes	A	G	E	G	S	I	R	N	L
TAYLOR *et al.*	2014		S	G	E	A	-	-	-	-	-
PEARCE *et al.*	2009		S	G	E	-	-	-	-	-	-
MOBULA *et al.*	2009		-	G	E	-	-	-	-	N	-
COHUET *et al.*	2006		-	G	E	-	-	I	R	N	-
SWARTHOUT *et al.*	2006		-	G	E	-	-	I	R	N	-
ALKER *et al.*	2008		S	G	K	-	-	I	R	N	-
BARAKA *et al.*	2017		S	G	E	A	-	-	-	-	-

Table 7| Distribution of mutations and haplotypes encountered on the K13 gene

Authors	Years of publication	Haplotypes K13 found
Mvumbi *et al.*	2017	F495L,Q476K,N523T,E509D
Taylor *et al.*	2014	R471R,G496G,R513R,V520A,A557S,V581V, A617T,V637A
Kamau *et al.*	2015	T493T,A578S

On the other hand, monitoring of targeted therapeutic combinations as alternatives showed the following failure rates: 0-20% for SP-AS, 1-32% for SP-AQ and 0-2% for AQ-AS [PNLP, 2009 -2013]. These results enabled the DRC to adopt the combination AQ-AS as a first-line drug in the treatment of simple malaria. Unfortunately in 2009, BONNET et al. published the in vivo test results conducted simultaneously at Kabalo and Boende of the AS-AQ and AL combinations. The conclusion shows that the AS-AQ CTA has a 15.1% resistance in Boende against 0% in Kabalo [BONNET et al., 2009].

Molecular analyzes carried out centered on the Pfcrt K76T, whose prevalence reached 88% and the CVIET haplotypes found in the proportions ranging from 55.4% to 100% [ANTONIA, 2014; SEVERINI, 2005], depending on the site. This shows that choloroquine is far from being reintegrated into our national malaria control policy. Genotyping showed high frequencies of dihydrofolatereductase (DHFR) and synthase dihydropteroate (dhps) molecular SP-resistance markers, with 57% of the samples exhibiting more than three mutations related to MS resistance, and 27% with triple -dhfr / double-dhps haplotypes, confirming that MS failure rates are likely to be elevated [SWARTHOUT et al., 2006]. MOBULA et al. [2009] found the molecular markers whose prevalences are the following pfmdr1 N86Y; Pfdhfr N51I, C59R and S108N; and pfdhps A437G gene were well above 50% and pfmdr1 Y184F, N1042D and D1246Y; Pfdhfr I164L; and pfdhps K540E gene were low.. Hence strict supervision is required. In the work of MVUMBI et al. [2017] three mutations (F495L, Q476K, N523T and E509D) were not detected in either Asia or Africa of mutations observed on the K13 gene.

Progress in the control of this endemic disease has been questioned in the past mainly due to the emergence of parasite resistance to antimalarial drugs [ASHLEY et al., 2014] . This has led most endemic countries to change their therapeutic protocols for artemisinin-based combination therapy (ACT), the best current treatments for uncomplicated Plasmodium falciparum malaria [ASHLEY, et al., 2014 *and* WHO, 2008] . The change in the therapeutic protocol is required if the resistance to an artemisinin-based molecule or combination is equal to or greater than 10%. The therapeutic efficacy tests for antimalarial drugs should be carried out every two years at sentinel sites but R.D. Congo, a large country of 2,345,409 km² and more 67,800,000 million inhabitants [EDS, 2010] shows that these studies on the therapeutic efficacy of antimalarials are difficult to implement especially in remote areas for various reasons Including: logistics, cost, geographical accessibility, insecurity.

This situation complicates the follow-up in the form of a framework set by the NMCP with the introduction of new antimalarial drugs and, at the same time, non-adherence by health care providers, lack of awareness of households in the national malaria control policy, lack of information among job sellers on the national therapeutic protocol and the circulation of counterfeit drugs further aggravate this situation [LOSIMBA, 2013; OKUNGU, 2014; WATSIERAH, 2012; KANGWANA, 2009; AMIN, 2007 *and* CALDERON, 2004] as the study in the Democratic Republic of Congo where 57% of the dihydroartemisinin (DHA), the active ingredient in artemisinin, was under dosage and this potentiated the development of resistance [ATEMNKENG et al., 2007] .

CONCLUSION

All these articles, although insufficient for a large country as DRC, show different resistance levels according to the study sites. This situation indicates that it is difficult to unify the same antimalarial treatment throughout the country. We propose monitoring resistance in the country as well as publication of all the studies to allow the real mapping of the resistance.

RÉSUMÉ

Résistance du Paludisme : une revue systématique de la résistance de *Plasmodium falciparum* aux antipaludiques en République Démocratique du Congo.

Le paludisme reste un problème majeur de santé publique en RDC et la principale cause de consultation, d'hospitalisation et de décès. Mais ces efforts sont susceptibles d'être effacés en augmentant l'émergence et la propagation de la résistance aux antipaludiques qui posent un grave problème de santé publique. Depuis 1983, lorsque des souches résistantes à la chloroquine ont été découvertes jusqu'à nos jours, quelles sont les différentes études sur la résistance du *Plasmodium falciparum* aux antipaludiques en République démocratique du Congo?

La recherche de cet examen a été menée en mars 2017 à l'aide des bases de données électroniques PubMed.gov, Yahoo et Google. Les termes de recherche «paludisme», «République démocratique du

Congo», «résistance», «antipaludique», «efficacité» ont été utilisés. Sur les 33 publications incluses, nous avons trouvé 11 articles sur le test *in vivo* seulement, 4 articles sur l'association test-*in-vitro in vivo*, 16 articles sur les marqueurs moléculaires seulement et 2 sur les marqueurs moléculaires et le test *in vivo*. Deux molécules antipaludiques (chloroquine, amodiaquine), six combinaisons de médicaments antipaludiques (sulfadoxine-pyriméthamine, artesunate-amodiaquine, arthemeter-lumefantrine, pyranadine-artesunate, dihydroartemisine-piperaquine et amodiaquine-sulfadoxine-pyrimethamine et cinq marqueurs moléculaires (pfcrt, Dhfr, k13) ont été évalués dans ces publications. Cependant, il est difficile de généraliser les résultats de ces études dans toute la République démocratique du Congo, car ces résultats sont différents d'un site à l'autre.

Après trente-quatre ans de la résistance du *Plasmodium falciparum* aux antimalariens en République Démocratique du Congo, il n'y a que trente-trois publications. Il est important d'augmenter les études et les publications sur la résistance palustre afin d'avoir toute la situation dans l'ensemble du pays.

Mots-clés: Résistance, paludisme, médicaments, marqueurs moléculaires, RDC

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Competing interests

The authors declare that they have no competing interests.

Authors' contributions

PNM and DNM conceived the study, and participated in its design and coordination. DNM supervised sample collection in the field and wrote the manuscript. PNM interpreted the data and wrote the manuscript. ALL and BSK carried out sample collection. PNM, JJTM and DNM participated in the discussions and reviewed the final manuscript. All authors read and approved the final version of the manuscript.

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