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Review

Epidemiology of malaria in Gabon: A systematic review and meta-analysis from 1980 to 2023

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ABSTRACT

The objective of this were conducted to elucidate spatiotemporal variations in malaria epidemiology in Gabon since 1980. For that, five databases, were used to collect and identify all studies published between 1980 and 2023 on malaria prevalence, antimalarial drug resistance, markers of antimalarial drug resistance and insecticide resistance marker. The findings suggest that Gabon continues to face malaria as an urgent public health problem, with persistently high prevalence rates. Markers of resistance to CQ persist despite its withdrawal, and markers of resistance to SP have emerged with a high frequency, reaching 100 %, while ACTs remain effective. Also, recent studies have identified markers of resistance to the insecticides Kdr-w and Kdr-e at frequencies ranging from 25 % to 100 %. Ace1R mutation was reported with a frequency of 0.4 %. In conclusion, the efficacy of ACTs remains above the threshold recommended by the WHO. Organophosphates and carbamates could provide an alternative for vector control.

Contents

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Introduction

Malaria is a vector-borne disease caused by protozoan parasites belonging to the *Plasmodium* genus. Nowadays, among six *Plasmodium* species (*Plasmodium falciparum*, *Plasmodium vivax, Plasmodium malariae, Plasmodium ovale*-wallikeri, *Plasmodium ovale*-curtisi and *Plasmodium knowlesi*) responsible for human malaria [1], *P. falciparum* is the most virulent and prevalent in Africa [2]. Malaria is a real public health problem, particularly in sub-Saharan Africa where the majority of cases are reported [3]. To fight this infection, from the late 1950s to the early 2000s, World Health Organisation (WHO) recommended chloroquine as the most effective treatment [4]. However, its overuse led to the emergence and spread of strains of *P. falciparum* resistant to this treatment, which prompted its withdrawal, particularly in Africa [5].

In Gabon, chloroquine (CQ) resistance was first described in 1983 [6]. By the early 2000s, it had spread to most of the country [7–9]. As a solution to the national resistance problem, chloroquine was prohibited in 2003, and the Gabonese malaria control strategy is now aligned with the 2001 recommendations of the WHO [10] which recommend artemisinin-based combination therapy (ACT) as first- and second-line treatment for uncomplicated malaria, intermittent preventive treatment with sulfadoxine-pyrimethamine for pregnant women, the use of long-lasting insecticide-treated nets (LLINs) and indoor residual spraying (IRS). The change in malaria control strategy reduced the burden of malaria in some urban areas of the country, notably in Libreville and Franceville, where the burden decreased from 31.2 % to 18.3 % between 2005 and 2008 [11] and from 69 % to 19.5 % between 2004 and 2009 [12], respectively. However, several studies have reported an increase in malaria cases in different regions of Gabon in recent years [11,13–16].

In addition, vector resistance to the various classes of chemical insecticides used in vector control (dichlorodiphenyltrichloroethane (DDT), pyrithrenoids, organophosphates and carbamates) is widely documented [17,18]. Mutations affecting the voltage-gated sodium channel gene, known as resistance knockdown (kdr), associated with resistance to DDT and pyrithrenoids [19,20], and mutations affecting the acetylcholinesterase insensitive gene (ace-1R), associated with resistance to organophosphates and carbamates [21] have been described in several regions of Africa [22–24]. In Gabon, studies have reported the presence of these mutations in various species of mosquito [25–27]. However, epidemiological data on malaria remain fragmented and do not allow visualizing overall trends throughout the country. Consequently, this systematic review and meta-analysis aimed to describe the spatio-temporal epidemiology of malaria prevalence, *P. falciparum* resistance to antimalarial drugs and mosquito resistance to insecticides in Gabon since 1980.

Methods

The analysis adhered to the recommendations of the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA, 2020) [28]. PRISMA is a 27-point checklist of preferred reporting items for systematic reviews (see Supplementary Table S1).

Literature search strategy

For this systematic review and meta-analysis, we conducted an extensive online literature search using a combination of keywords. We searched through various databases, including PubMed, Google Scholar, CrossRef, ProQuest, and WWARN, to identify relevant articles. We also searched for articles available in different languages using the following search terms: "Gabon AND malaria", "Gabon AND anti-malarial AND in vitro", "Gabon AND antimalarial AND in vivo", "Gabon AND antimalarial AND efficacy", "Gabon AND malaria AND drug resistance", "Gabon AND anopheles" and "Gabon AND insecticide resistance" were searched. Additional searches were conducted using references identified in primary reports to expand the eligible studies. Corresponding authors were contacted to obtain full-length texts for articles with restricted access.

Eligibility criteria

Inclusion criteria

In this review includes all malaria studies conducted in Gabon between January 1980 and October 2023, written in English or French, and focused on four areas of study: malaria prevalence, therapeutic efficacy (in vitro and in vivo) of anti-malarial drugs such as Chloroquine (CQ), Sulphadoxine-pyrimethamine (SP), and Artemisinin-based Treatments (ACT) based on the protocol defined by the WHO. Also, the studies on molecular markers of anti-malarial drug resistance, including *Pf*crt, *Pf*mdr1, *Pf*dhfr, *Pf*dhps, *Pf*cytb, *Pf*atp6 and *Pf*k13. Additionally, it examines marker of vector resistance to insecticides, such as Kdr-e, Kdr-w and Ace 1 R. The study also included studies of malaria cases imported from Gabon and multicentric studies in which Gabon was one of the study sites.

Exclusion criteria

Abstracts were excluded, as well as studies on malaria prevalence or antimalarial drug resistance without epidemiologic data. Additionally, studies on species other than *P. falciparum*, studies on the genetic diversity of *P. falciparum*, in vivo studies evaluating antimalarial drug combinations other than ACT, and clinical trials that administered antimalarial drugs at doses lower than the recommended standard dose were also excluded.

Selection process

The eligibility of research articles identified from the online literature search was determined by screening their titles and abstracts. Ineligible studies, including duplicates and those that did not meet the inclusion criteria, were removed. Selected articles were fully read to confirm their eligibility before data extraction, except for the two articles for which we were unable to obtain the full text

[6,29]. Eligible studies were selected by two independent reviewers, YVSB and SSO, who screened the titles and abstracts. Any disagreements between the evaluators (YVSB and SSO) were resolved by the intervention of a third evaluator, LB. No ethical approval or informed consent was required to include articles involving human subjects.

Data extraction process

A structured data collection process is required to address possibility of confusing or missing data. A data extraction form was designed using Microsoft Excel 2016 (Microsoft Corporation, United States of America). Two authors independently (YVSB and SSO) worked to extract the dataset. In cases where there were discrepancies in article selection, two other authors acting as independent reviewers NMLP and LB were consulted to resolve the issue. The entire process was overseen by two senior researchers, JBLD and LB. Specific data of all studies consisted of the lead author's name, publication date, study duration, study location, and the target population. We collected data on malaria prevalence, target populations, sources of the population, and recorded malaria cases. Malaria prevalence was calculated based on the ratio of positive cases to the total number of individuals screened.

If multiple diagnostic tests were conducted on the same individuals, we considered the results of the most sensitive test first; PCR > Microscopy > RDT. Children were defined as those aged 15 years or younger, while pregnant women were classified from the first month of pregnancy to the day of delivery. If a study lasted at least two years and did not report malaria cases by year, the year of the study started was considered to be the study year. Regarding data on in vitro, we recorded the name of the tested antimalarial drug, the testing protocol, the number of isolates tested, the number of resistant isolates, the observed prevalence of resistance, and the antimalarial drug resistance threshold.

For in vivo test, we collected information on the number of patients under observation, the specific antimalarial drug tested, the total drug dosage administered, the treatment duration, the follow-up duration, the number of patients experiencing therapeutic failure, the therapeutic failure rate within the studied population, and whether molecular genotyping was employed to differentiate recrudescence from reinfection. the failure rate was calculated based on per-protocol patients. Parasitological failures were categorized as treatment failures, regardless of symptoms. In terms of data on molecular markers of antimalarial drug resistance, we documented the gene analyzed, details of the mutated allele, the number of sequencing-positive isolates, the sequencing technique used, the number of mutated isolates, and the prevalence of the mutation. Mixed genotypes were considered mutants as described elsewhere [30], and dhfr-dhps quadruple and quintuple mutations with IRNG and IRNAG, as major haplotypes [31]. Lastly, for data related to insecticide resistance, we recorded the number of Anopheles species involved, the target gene under analysis, the sequencing technique used, details of the mutated allele, and the frequency of resistance.

Data items

The study eligibility criteria were determined using the PICOS format. This includes the following: Population (P): patients of any age (children or adults), any sex (men or women), infected with P. falciparum, whether asymptomatic or symptomatic, uncomplicated, or severe malaria. Intervention (I) or exposure (E): Patients diagnosed with malaria by rapid diagnostic test (RDT), microscopy, or PCR. The therapeutic efficacy of chloroquine (CQ), sulfadoxine-pyrimethamine (SP), or artemisinin-based combination therapies (ACT). Comparator (C): None. Outcome (O): prevalence of *P. falciparum* in diagnosed populations, therapeutic efficacy rates of CQ, SP and ACT in patients tested, prevalence of molecular markers to antimalarial drugs. The study design includes observational studies, cross-sectional studies, case reports, and cohorts, and interventional studies such as randomized controlled trials.

Study risk of bias assessment

To assess potential bias in the included studies, we used the revised Cochrane Collaboration tool (RoB 2.0) to classify randomised clinical trials as having a low, high or uncertain risk of bias [32]. Cohort studies were evaluated using the Newcastle-Ottawa scale to assess selection bias, comparability, and evaluation of results. This rating scale classifies cohort studies as unsatisfactory (0 to 3 points), satisfactory (4 to 5 points), good (6 to 7 points), or very good (8 to 9 points) [33]. Finally, the quality of cross-sectional studies was evaluated using the Joanna Briggs Institute (JBI) critical appraisal checklist [34]. The Joanna Briggs Institute classifies the risk of bias assessment for cross-sectional studies as follows: studies meeting at least 75 % of the quality criteria are classified as low risk of bias, studies meeting between 50 % and 74 % of the quality criteria are classified as moderate risk of bias, and studies meeting less than 49 % of the quality criteria are classified as high risk of bias (see additional files 1, 2, 3 and 4 provide further details). The risk of bias in the included studies was independently assessed by two reviewers, YVSB and SSO. Any differences of opinion regarding the risk of bias were resolved through discussion by the evaluators.

Synthesis methods

The meta-analyses were performed using R software (version R x 64.3.6.2). A random-effects model was used to assess heterogeneity among the studies included in the final meta-analysis, taking into account the total number of screened studies and the number of positives results for both malaria prevalence and for each individual molecular marker of antimalarial drug resistance (See supplementary Fig. S1, Fig. S2). The I^2 statistic, also known as the inconsistency index, was used to indicate the percentage (%) of heterogeneity that could be attributed to the variance between studies. An I^2 value of 25 % indicates low heterogeneity, 50 % indicates moderate heterogeneity, and 75 % indicates high heterogeneity [35]. To assess the possibility of publication bias, we examined the asymmetry of the funnel plots and conducted Egger's regression test. The funnel plot displays the proportion on the x-axis and the standard error on the y-axis (See supplementary Fig. S3-S9).

The data on malaria distribution and molecular markers of resistance were stratified based on the study period. The spatial distribution of malaria was divided into three periods: 1992–2004, 2005–2015, and 2016–2021. To visually represent the prevalence of malaria, we created maps using QGIS software (QGIS Development Team, 2022) and Adobe Illustrator CS3 to enhance and combine the images obtained. To determine the prevalence of malaria in each province, we compiled the results of malaria positivity tests conducted on the selected population in each provincial study. The temporal evolution of molecular markers of anti-malarial drug resistance was divided into three periods: 1995–2003, 2004–2011, and 2012–2020. The findings were presented in a frequency table, and a map was used to illustrate their spatial distribution. Statistical tests, such as chi2 or Fisher's exact test, were used to compare changes in prevalence and point mutations over time. All statistical analyses were performed at a significance level of 5 % (p-value < 0.05) and a confidence interval (CI) of 95 %.

Role of the funding source

The funder had no role in the study design, data collection, analysis, interpretation, or writing of the report.

Results

Study characteristics

We included 386 studies that were deemed eligible for full-text examination, among them 162 were retained. Seventy-three (45.1 %) focused on malaria prevalence, 17 (10.5 %) delved into in vitro antimalarial drug resistance, 25 (15.4 %) centred on in vivo tests, 40 (24.7 %) examined molecular markers of antimalarial drug resistance, and 7 (4.3 %) investigated markers of insecticide resistance (Fig. 1 and Supplementary study characteristics).

Of the 73 studies analysed on the prevalence of malaria, the majority (28) were carried out in the Estuaire province, followed by the Haut-Ogooué and Moyen-Ogooué provinces, with 19 studies each. We observed that the Ogooué Maritime province was the least studied, with three studies. After examination, we observed that the number of studies varied over time by including different locations (Supplementary Table S2). We observed that the studies evaluating in vitro efficacy were conducted in three provinces (Estuaire (3), Haut-Ogooué (5), and Moyen-Ogooué (9), with most using the WHO isotope microtest or semi-microtest (Additional file 2). The majority of in vivo resistance studies were conducted in Lambaréné , in the Moyen-Ogooué province (n = 14), with others in the Estuaire (n = 4), Haut-Ogooué (n = 3), and Woleu-Ntem provinces and some being multicentre collaborations (n = 3) (Additional file 3). With regard to antimalarial drug resistance markers, the main markers studied were as follows: *Pfcrt* (15), *Pfmdr1* (16), *Pfdhfr*, *Pfdhps*, *Pfatpase6*, *Pfcoronin* and *Pfcytb* (Additional fine 4).

and *Pfk13* (7) (Table 1). With regard to vector resistance, three studies looked at the Ace1R allele, five at the Kdr-e allele and seven at the Kdr-w allele (Table 2).

Temporal distribution of malaria in Gabon

From 1992 to 2021, a total of 165,642 individuals of all ages underwent screening for *Plasmodium* parasite infection. A total of 57,630 confirmed malaria cases were documented across all nine provinces. Outside of the 1992–2021 period, no reliable and available data on Plasmodium screening were found at the date of the search. *Plasmodium* spp. infection was significantly more prevalent among febrile cases (36.1 %, 50,461/139,839) compared to afebrile cases from the general population (27.8 %, 7170/25,803), $p < 0.001$ (Fig. 2A). From 1992 to 2006, the average malaria prevalence stood at 36.3 % before declining to 23.4 % between 2007 and 2011. From 2011, data indicated an upsurge in the number of *Plasmodium* malaria infection cases within the population, reaching a peak prevalence of 68.5 % in 2015 before declining gain from 2016 onwards, although the prevalence remains relatively high around 35 % (Fig. 2B).

In pregnant women, *Plasmodium* infection was detected in 17.0 % of the women screened (975/5731), with a significant difference in the proportion of pregnant women positive for *Plasmodium* before and after 2005. Between 1995 and 2004, the average malaria infection prevalence was 31.4 %, which then significantly decreased to 11.4 % by 2005 (Fig. 2C). For children, malaria prevalence was 33.7 %, exhibiting fluctuations over time. It ranged from 35.5 % from 1992 to 2004, declining to 21.0 % between 2005 and 2010, before rising to 44.3 % between 2013 and 2018 (Fig. 2D).

Spatial distribution of malaria in Gabon

Based on the available data, malaria parasite infection is not evenly distributed throughout the country, and the prevalence of the disease has varied from province to province over the course of the study period (Fig. 3). From 1992 to 2004, taking into account the unavailability of some of the data, the Estuaire and Haut-Ogooué provinces were hotspots of infections (Fig. 3A). Between 2005 and 2015, the Haut-Ogooué province had the highest prevalence rate at 54.5 % (Fig. 3B), compared to 46.5 % between 1992–2004 and 28.7 % in 2016–2021 (Fig. 3C). In the Ogooué -Lolo province, the prevalence rate increased from 26.5 % in 2005–2015 to 43.1 % in 2016–2021 (Fig. 3C). More recently, the highest prevalence rates are found in the Woleu-Ntem, Ogooué -Ivindo, and Haut-Ogooué provinces, which representing the north, northeast, and southeast regions, respectively. In contrast, the lowest prevalence rates are found in the coastal regions of the Ogooué -maritime and Nyanga provinces, located in the west and south, respectively (Fig. 3D). The central zone that runs diagonally across the country and which includes the provinces of Estuaire, Moyen-Ogooué , and Ngounié , can be considered an area of moderate malaria transmission (Fig. 3D).

Molecular markers of antimalarial drug resistance have been described in most regions of the country, according to our findings. In Lambaréné , the prevalence of the *Pfcrt* 76 T allele was 100 % between 1996 and 2002 [36–39]. Similar levels were documented in Franceville in 2004 (95.8 %) and 2009 (97.8 %) [40], Bakoumba (94 %) in 2010 [41], and Oyem (82 %) in 2005 [42]. The prevalence of the *Pfmdr1* gene's 86Y and 184 F alleles was reported to be high in Lambaréné (86Y-93 %) during 1995/6 [43] and 2002 [38], in Oyem in 2005 (86Y-86.2 %) [42], and in Bakoumba (184 F-96 %) in 2010 [41]. The 1246Y allele was present below 50 % in all areas except for Dienga, where it ranged between 54 % and 74 % from 2013 to 2014 [44]. High prevalences of three *Pfdhfr* alleles, namely 51I, 59 R and 108 N, were observed in Oyem (108 N-100 %) in 2005 [45], Bakoumba (51I-91 %) in 2010 [41], Libreville [51I,59 R,108 N (100 %)] in 2011 [46], and finally Fougamou [51I,108 N (100 %)] in 2016 [47]. For the *Pfdhps* gene, the prevalence of the 437 G allele was highest in Libreville (94.7 %) and Franceville (95.7 %) between 2014 and 2018 [48], as well as in Fougamou (100 %) in 2016 [47]. In contrast, the prevalences of the other alleles, namely 436 A, 540E and 613 S, have remained relatively stable (Fig. 4A).

The highest occurrence of CVIET haplotypes of *Pfcrt* was recorded in Franceville (97.8 %) during 2009–2010 [49], as well as in Koula-Moutou (96.3 %) and Lastoursville (96.1 %) in 2014 [50]. Earlier than that, the highest levels of the *Pfdhfr* IRN triple mutant were found in Libreville (80 %) between 2005 and 2006 [51], Lambaréné (92 %) between 2005 and 2007 [52], and Oyem (91.9 %) in 2008 [45]. Quadruple mutations of IRNG were identified frequently in various regions of Gabon, including Franceville in 2013 and 2014 where they were found at 100 % and 89 %, as well as Koula-Moutou (91.7 %), Lastoursville (100 %) in 2014 [50], and Fougamou (93.1 %) in 2016 [47]. Conversely, quintuple mutations were primarily detected in different region of Gabon for example in Lambarene (5.1 %) between 2005 and 2007; Estuaire the frequency varied between 7.9 % to 61 % in period of 2005 to 2019 and in Fougamou the frequency was 31 % (Fig. 4B).

Analysis of changes in molecular markers between 1995 and 2020 showed a significant reduction in the 76 T allele, from 99.8 % to 20.9 % ($P < 0.0001$). In addition, there was a decrease in alleles 86Y and 184 F, whereas allele 1246Y indicated an increase from 5.3 % to 23.6 % ($P < 0.0001$). Considerable increases were seen in alleles 51I, 59 R, and 108 N, while the proportion of allele 436 A dropped remarkably from 28.1 % to 19.9 % ($P = 0.003$). During the time frame studied, there was a remarkable increase in the proportion of the 437 G allele, rising from 41.5 % to 86.9 % ($P < 0.0001$), alongside a significant growth in the 540E allele, increasing from 0 % to 32.2 % ($P < 0.0001$). However, the A581G and A613S alleles did not exhibit any noticeable changes during the given period (Supplementary Table s3). The haplotypes CVIET, IRN, IRNG, and IRNAG showed a significant increase (Supplementary Table s3).

The examination conducted identified a total of seventeen single-nucleotide polymorphisms (SNPs) in the *Pfk13* gene [31,50,53–57]. Only one case (S522C) was reported in this study, but at a low frequency of 0.2 % (Table 1).

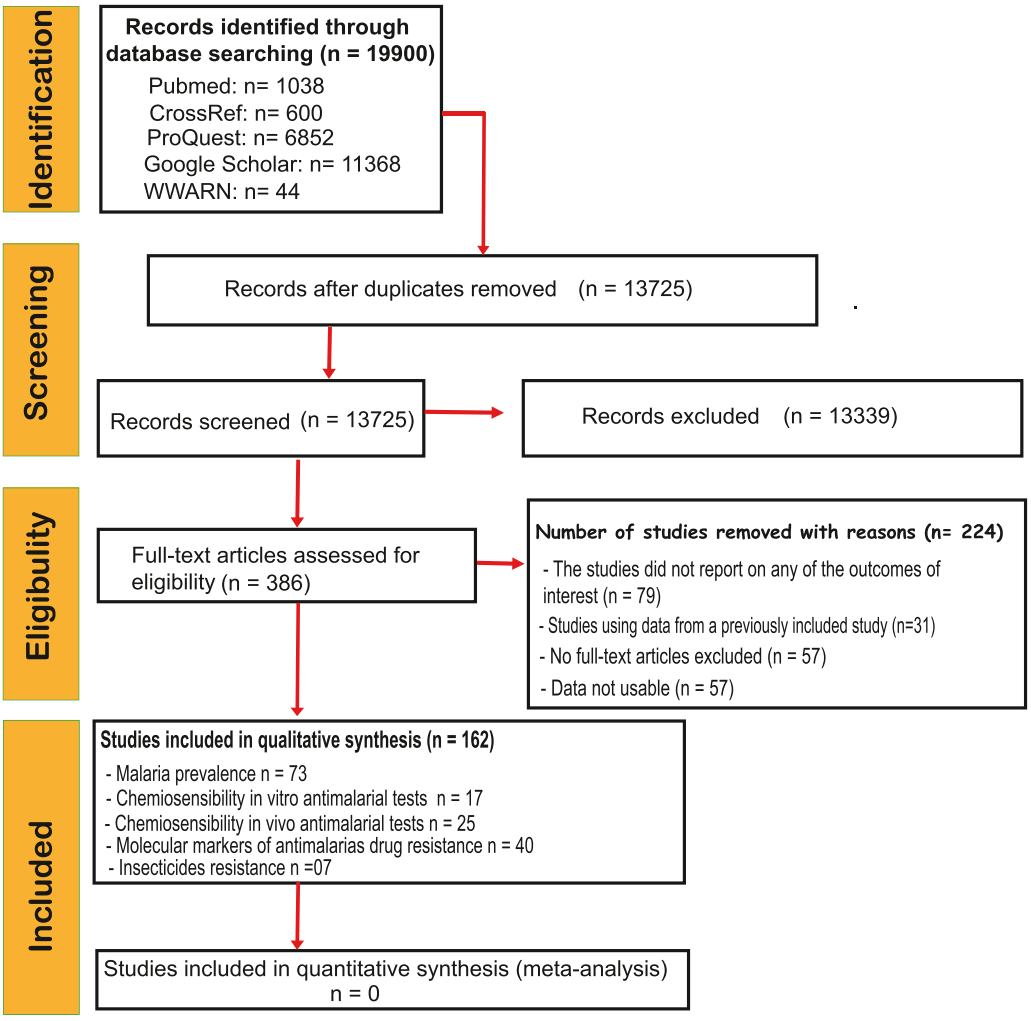

Fig. 1. Selection process for systematic review.

Table 1
Frequency and distribution of polymorphism in the *Pfkelch13* gene in Gabon.

Author's name	Year of collection	Site	Mutation name	Type of mutations	Mutation frequency %, (n/N)
Kamau et al.,2015	2007	Libreville	R471R	Synonymous	1.08 (1/93)
Kamau et al.,2015	2007	Libreville	A578**S**	Non-synonymous	1.08 (1/93)
Kamau et al.,2015	2007	Libreville	L589I	Non-synonymous	1.08 (1/93)
Voumbo-Matoumona et al.,2018	2011	Franceville	V494A	Non-synonymous	0.4 (1/233)
Voumbo-Matoumona et al.,2018	2011	Franceville	A504V	Non-synonymous	0.4 (1/233)
Voumbo-Matoumona et al.,2018	2011	Franceville	A578S	Non-synonymous	0.4 (1/233)
Nguetse et al.,2017	2012	Lambaréné	V589I	Non-synonymous	0.9 (1/114)
Nguetse et al.,2017	2012	Lambaréné	V589V	Synonymous	0.9 (1/114)
Nguetse et al.,2017	2012	Lambaréné	N645N	Synonymous	0.9 (1/114)
Voumbo-Matoumona et al.,2018	2013	Koula-Moutou	V494A	Non-synonymous	0.4 (1/233)
Ménard et al., 2016	2014	Libreville	V454A	Non-synonymous	0.24 (1/416)
Ménard et al., 2016	2014	Libreville	N499S	Non-synonymous	0.24 (1/416)
Ménard et al., 2016	2014	Libreville	S522C*	Non-synonymous	0.24 (1/416)
Ménard et al., 2016	2014	Libreville	G625R	Non-synonymous	0.24 (1/416)
Leroy et al.,2019	2014–2015	Libreville	A578**S**	Non-synonymous	2.4 (2/83)
Kayiba et al.,2021	2016	Libreville	S522C*	Non-synonymous	0.2 (1/407)
Dinzouna-Boutamba et al.,2023	2019	Libreville	Y493H*	Non-synonymous	0 (0/58)
Dinzouna-Boutamba et al.,2023	2019	Libreville	R539T*	Non-synonymous	0 (0/58)
Dinzouna-Boutamba et al.,2023	2019	Libreville	I543T*	Non-synonymous	0 (0/58)
Dinzouna-Boutamba et al.,2023	2019	Libreville	A578S	Non-synonymous	0 (0/58)
Dinzouna-Boutamba et al.,2023	2019	Libreville	C580Y*	Non-synonymous	0 (0/58)
Dinzouna-Boutamba et al.,2023	2019	Libreville	V589I	Non-synonymous	0 (0/58)
Dinzouna-Boutamba et al.,2023	2019	Libreville	E/G 605 K	Synonymous	0 (0/58)

* Molecular marker for artemisinin resistance validated by the WHO

Table 2
Changes in the frequency of insecticide resistance markers from 1999 to 2021.

Autor's name	Year of study	Site	N	Gene (Allele)	RR	RS	SS	FR (%)
Santolamazza et al.,2008	1999	Benguia	57	kdr-w (L1014F)	Not specified	Not specified	Not specified	6.1
Santolamazza et al.,2008	1999	Benguia	57	kdr-e (L1014S)	Not specified	Not specified	Not specified	7
Pinto et al.,2006	2000	Libreviile	106	kdr-w (L1014F)	10	0	0	37
Pinto et al.,2006	2000	Libreviile	106	kdr-e (L1014S)	37	0	0	63
Mourou et al.,2010	2006-2007	Libreviile	250	kdr-w (L1014F)	80	1	0	59.4
Mourou et al.,2010	2006-2007	Libreviile	250	kdr-e (L1014S)	33	0	0	40.3
Mourou et al.,2010	2006-2007	Libreviile	250	Ace 1 R (G119S)	0	2	248	0.4
Mourou et al.,2010	2006-2007	Port-Gentil	50	kdr-w (L1014F)	21	1	0	61
Mourou et al.,2010	2006-2007	Port-Gentil	50	kdr-e (L1014S)	3	0	0	31
Mourou et al.,2010	2006-2007	Port-Gentil	50	Ace 1 R (G119S)	0	0	50	0
Mourou et al.,2012	2008-2010	Libreviile	1020	kdr-w (L1014F)	597	2	0	76
Mourou et al.,2012	2008-2010	Libreviile	1020	kdr-e (L1014S)	61	1	0	23.5
Mourou et al.,2012	2008-2010	Libreviile	1020	Ace 1 R (G119S)	0	0	0	0
Koumba et al.,2018	2017	Mouila	234	kdr-w (L1014F)	231	3	0	99
Koumba et al.,2018	2017	Mouila	234	kdr-e (L1014S)	33	26	175	20
Koumba et al.,2018	2017	Mouila	234	Ace 1 R (G119S)	0	0	234	0
Boussougou-Sambe et al.,2022	2017-2018	Lambaréné	116	kdr-w (L1014F)	67	0	0	73.9
Boussougou-Sambe et al.,2022	2017-2018	Lambaréné	116	kdr-e (L1014S)	10	0	0	25.2
Boussougou-Sambe et al.,2022	2017-2018	Lambaréné	116	Ace 1 R (G119S)	0	0	116	0
Longo-Pendy et al.,2022	2021	Libreville	30	kdr-w (L1014F)	28	2	0	96.7
Longo-Pendy et al.,2022	2021	Cocobeach	19	kdr-e (L1014S)	19	0	0	100

N: number of anopheles species analysed; **RR**: resistant homozygotes; RS: heterozygotes for resistance; SS: Susceptible homozygotes; FR: Frequency of the resistance allele.

Frequency and distribution of alleles involved in resistance to DDT and pyrethroids in Gabon

Between 1999 and 2021, 1882 samples of *Anopheles gambiae spp*. were studied to investigate insecticide resistance markers, including kdr-w, kdr-e, and ace1R. The first recorded insecticide resistance markers in mosquitoes were found in Libreville in 2000 [58], where high rates of Kdr-w and Kdr-e resistance alleles were observed, with frequencies of 37 % and 67 %, respectively. This resistance then spread to other regions in the country, including Benguia [59], Port-Gentil [60], Mouila [26], and Lambaréné [27]. Over time, there has been a consistent increase in the prevalence of both kdr-w and kdr-e alleles, with the kdr-w allele exhibiting higher frequencies than kdr-e. It is important to note that only one study has reported the presence of the Ace1R mutation, which was found at a relatively low frequency of 0.4 % (Table 2).

Discussion

Acquiring up-to-date and comprehensive epidemiological data is crucial to improve malaria control measures and potentially achieve complete eradication. Evaluating the changes in malaria prevalence over time and mapping its geographical distribution within the country and among different populations are critical for assessing the impact of national-level protocols and interventions aimed at malaria control. In this study, we extensively reviewed published literature spanning the past four decades to create a nationwide map of malaria prevalence and its temporal evolution in Gabon. The necessity for this approach arises from the paucity of local, provincial, and national data on malaria prevalence and the identification of high-risk populations and regions. The absence of a comprehensive national surveillance or health information system often results in incomplete and outdated data on the malaria burden. In Gabon, malaria prevalence data are collected in a fragmented and sporadic manner, primarily within public health facilities in major cities. However, it is widely acknowledged that the regular collection of malaria data is fundamental to monitoring the disease burden and formulating and implementing effective health policies for malaria control. We chose to collect data on malaria prevalence from 1992 onwards due to the lack of available studies in the databases we consulted prior to this year. The studies we reviewed accurately reflect the research conducted during the review period. Our findings unveiled an average prevalence of malaria infection at 34.8 % (ranging from 17.4 % to 75.4 % at the national level, with fluctuations over time. Malaria prevalence decreased from 36.3 % between 1992–2006 to 23.4 % between 2006 and 2011. The decrease in malaria cases in Gabon can be credited to following the WHO guidelines, especially after 2003 when a new policy for malaria treatment and prevention was introduced. This policy mandated the use of ACTs for treating malaria and required pregnant women to receive intermittent preventive treatment (IPTp) starting from their second trimester. Other measures like distributing insecticide-treated bed nets and indoor residual spraying were also put in place. However, the complete implementation of these interventions across the entire country did not happen until 2005. Similar progress has been observed in other African countries like Rwanda, Ethiopia, and Mozambique [61–63]The effects of policy changes are particularly notable among vulnerable groups, including pregnant women and children. For pregnant women, there was a clear decrease in malaria infection rates after the introduction of new policies, with convincing evidence of high adherence to preventative measures [64,65]. This trend appears to have been maintained to date, in both urban and rural areas. However, among the rest of the population, especially children, there has, unfortunately, been a significant increase in *Plasmodium* malaria infection rates since 2011, possibly due to relaxed prevention efforts. This is consistent with the results of the last two Demographic and Health Surveys (2012 and 2019–2021), which showed limited access to long-lasting insecticide treated nets (LLINs) for vulnerable populations in both urban and rural areas, and low household coverage with LLINs, which has even decreased over the last decade, from 36 % in 2012 to 21 % in 2019–2021). [66,67]. In addition, there is quasi non-use of indoor residual spraying. This overall trend in the prevalence of malaria infection over time and the impact of public policies on malaria prevention and control is not reflected in the same way in the country's different regions. Over time, changes can be observed in the spatial distribution of the main hotspots of malaria parasite infection, reflecting the trends observed at the provincial level over the same period. These changes may reflect the non-uniform application and varying intensity of the malaria prevention and control programs implemented. Indeed, availability and coverage of LLINs has been shown to vary widely between provinces [67]. Socio-demographic, socio-economic, environmental, and climatic trends may explain at least part of these changes. Similar observations have been made elsewhere in Africa [68,69]. Nevertheless, it is important to note that the transmission cold spots (coastal regions) were remained relatively stable. The

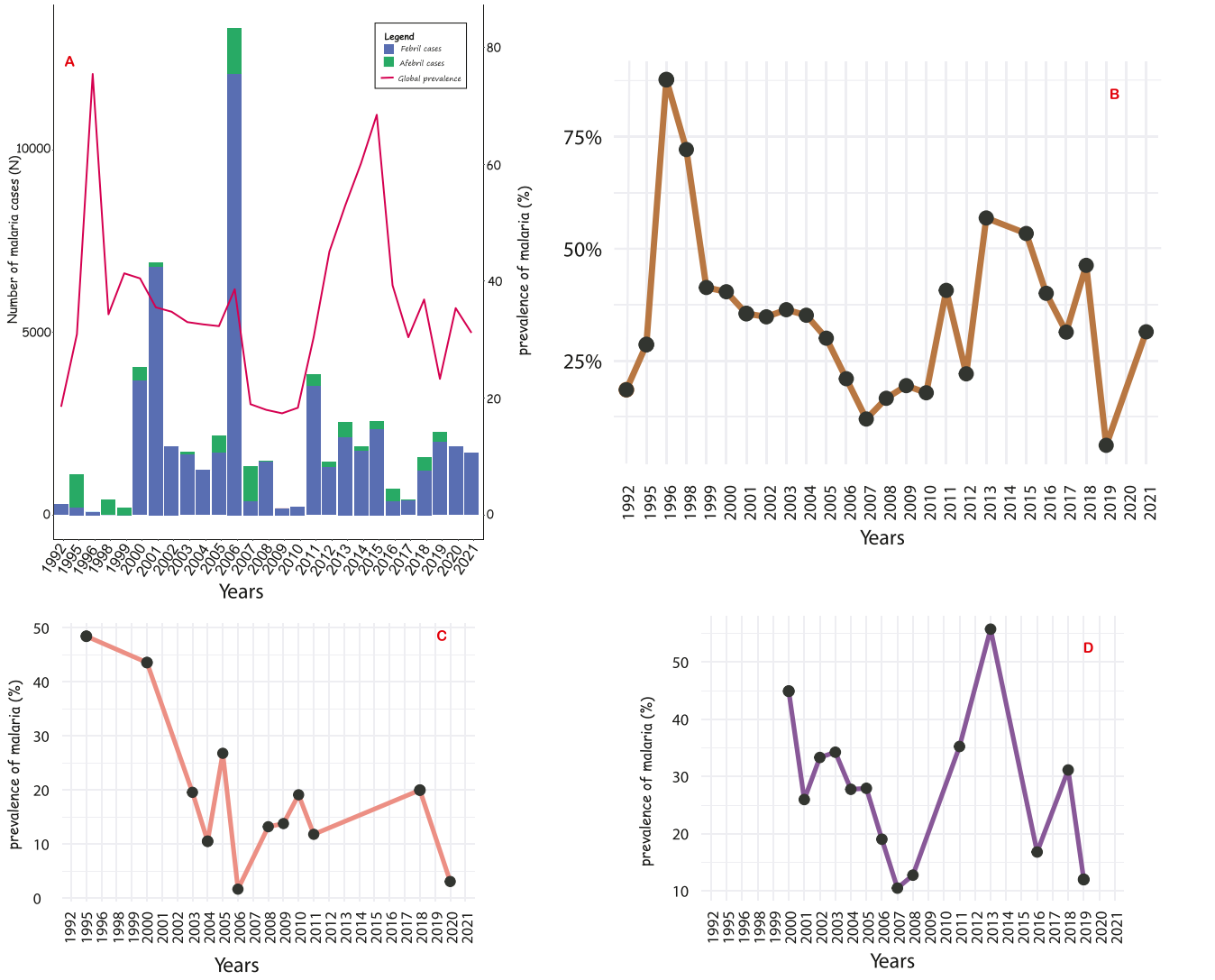

Fig. 2. Trend in malaria prevalence over time in Gabon from 1992 to 2021. 2 A: Temporal evolution of the number of malaria cases (febrile vs afebril) against the global prevalence. 2B: Temporal evolution of the prevalence of malaria in the general population.

overlay of global spatial and temporal malaria prevalence data reveals a non-random distribution of malaria transmission across the country. An epidemiological gradient (ranging from low, medium, and high) extends from the west coast to the eastern international borders, closely mirroring the intensity of forest cover. The forest ecosystem provides ideal conditions for vector proliferation, sustaining disease transmission dynamics [70]. In summary, the temporal and spatial data suggest that Gabon continues to be a mesoendemic country for malaria, although the eastern part of the country has shown a strong hyperendemic tendency. The trends observed over the last decades indicate a sustained prevalence of infection at relatively high levels [11,13–16,71,72]. In addition to the factors mentioned above, the resistance of *Plasmodium* to antimalarial drugs remains a significant contributing factor to this situation.

In Gabon, the first signs of resistance to chloroquine emerged in 1983 (Fig. 5), starting in Lambaréné [6] and later spreading to various regions across the country [8,9,29,37,73–77]. Molecular studies have shown that CQ resistance markers circulate at high frequencies in Lambaréné [36–39], Bakoumba [41], Franceville [40], and Oyem [42,78] As a result, chloroquine was abandoned in favour of ACTs [10]. This resistance spread can be attributed to various factors, including the unrestricted sale of antimalarial drugs in pharmacies, leading to their misuse, poor adherence to treatment regimens, and the practice of self-medication [7,79]. To our knowledge, there are currently no measures in place in the country to mitigate these issues, emphasizing the need for strategies to reduce inappropriate antimalarial drug use through national policies. However, despite the withdrawal of CQ, in vitro studies carried out in 2009 and 2015 uncovered *P. falciparum* strains that were resistant to it [49,80]. These findings contrast those reported in Malawi, where chloroquine eliminated 100 % of *P. falciparum* infections without in vitro resistance 8 years after its withdrawal [81]. Another study conducted in the same country reported 99 % in vivo efficacy of chloroquine [82]. Furthermore, although there was a significant decrease in the frequency of the 76 T allele between 1995 and 2020 in Gabon, it remains higher than the rates reported by other authors, particularly in Malawi [81], Zambia [83], and Tanzania [84] This could be accounted for by the fact that in these regions, the discontinuation of chloroquine was succeeded by the implementation of sulfadoxine-pyrimethamine as the primary treatment before the transition to artemisinin-based combination therapies. In contrast, in Gabon, the discontinuation of chloroquine was followed by the use of amodiaquine as monotherapy between 2003 and 2005/6, and then in conjunction with artemisinin derivatives [42]. The mode of action of these two antimalarial drugs appears to be similar, and cross-

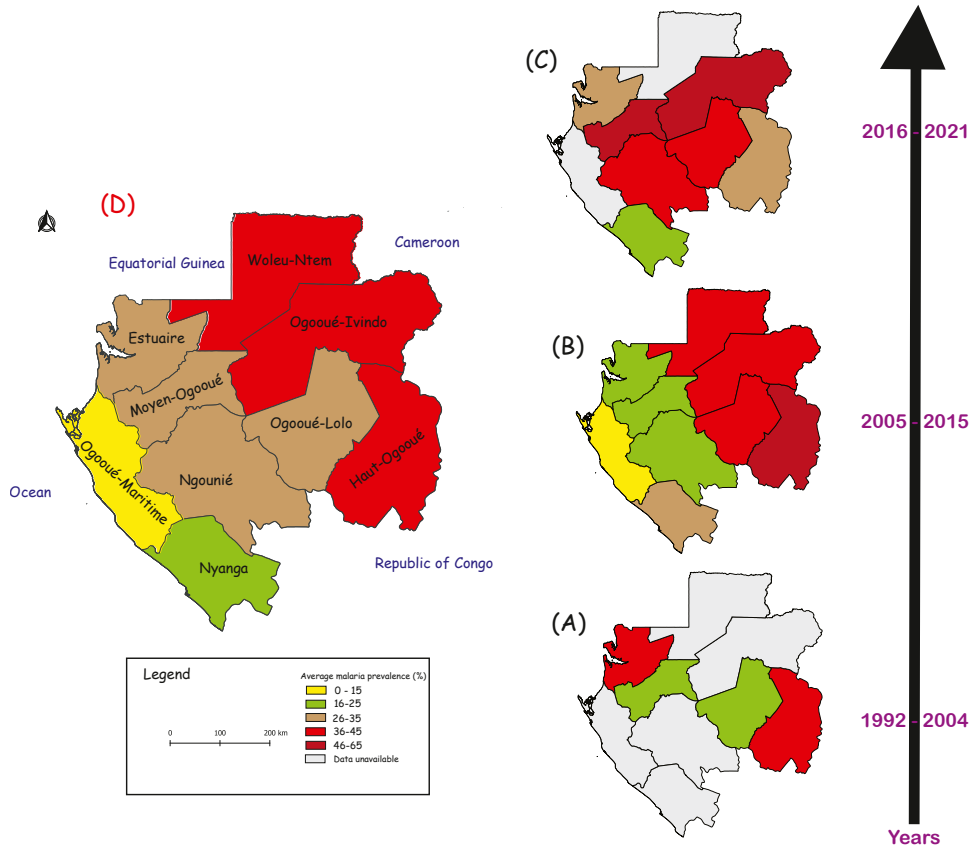

Fig. 3. Spatial distribution of malaria prevalence in Gabon from 1992 to 2023. 3 A: spatial distribution between 1992 and 2004; 3B: spatial distribution between 2005 and2015; 3 C: spatial distribution between 2016 and 2021; 3D: spatial distribution in 1992 to 2021.

resistance in vitro and in *vivo* has been widely documented [85–89]. The utilization of amodiaquine may potentially maintain the existence of the 76 T allele within the population. Polymorphisms in the 86Y, 184 F and 1246Y alleles of the *Pfmdr1* gene have been associated with reduced sensitivity to amodiaquine [90], mefloquine [91] and lumefantrine [92,93]. High frequencies of these alleles were observed in Lambaréné [36,38,43], Bakoumba [41]and Oyem [42]. These results are consistent with the fact that amodiaquine, mefloquine and lumefantrine are used in combination with specific artemisinin derivatives for the treatment of uncomplicated malaria [42,94]. However, the data show that the 86Y and 184 F alleles decreased over time, while the 1246Y allele increased and the S1034C and N1042D alleles remained relatively stable. A shift to dual therapy treatments has been embraced to address the resistance challenges encountered with most monotherapy treatments as a more effective approach in malaria management. This transition is exemplified by sulfadoxine-pyrimethamine (SP), officially introduced as a preventive treatment for pregnant women in 2005. However, reports of in vitro and in vivo resistance [72,95–99] to SP had already been widespread since the first documented case in 1988 [100]. This resistance can be attributed to the frequent use of SP in regions of the country where chloroquine resistance was prevalent. The most recent in vivo study, conducted between 2005 and 2007, indicates a failure rate of 54 % for SP [52]. Furthermore, molecular data have highlighted the association between SP resistance and the *Pfdhfr* and *Pfdhps* genetic markers. These markers have exhibited high-frequency mutations in many rural and urban areas across the country, including Libreville, Oyem, Koulamoutou, and Fougamou [45–47]. These high mutation frequencies align with findings from other regions, such as in the Bata district and on the island of Bioko in Equatorial Guinea, Cameroon [101] and Niger [102]. Overall, there has been a decrease in the 436 A allele and an increase in the 51I, 59 R, 108 N, 437 G, and 540E alleles, as well as in the IRN, IRNG, and IRNAG haplotypes, while the 581 G and 613 S alleles have remained relatively stable between 1995 and 2020. These frequencies are probably a consequence of the increased use of sulfadoxine-pyrimethamine among pregnant women. This results in a selection pressure which favours the development of SP-resistant parasites in Gabon and many other sub-Saharan African countries where SP is employed as a preventive treatment for pregnant women. Nevertheless, numerous studies have demonstrated the efficacy of SP in reducing the prevalence of *Plasmodium* infections in pregnant women in Gabon [103–105]. Further studies on the phenotype correlated with resistance to SP are needed to confirm the high frequencies observed. Current data indicate that artemisinin-based combination therapies (AL and AS-AQ) maintain an efficacy rate of almost 95 % [106–108]. Resistance to ACTs has already been documented in other regions, particularly Southeast Asia [109] and specific areas within sub-Saharan Africa [110,111]. Multiple single-nucleotide polymorphisms in the *Pfk13* propeller region have been linked to delayed parasite clearance following antimalarial treatment [112–114]. One SNP (S522C) identified in this study were previously validated as artemisinin-resistant by the World Health Organization (WHO). This highlights the need for further studies to monitor the efficacy of these treatments and facilitate robust epidemiological surveillance. Apart from *Plasmodium falciparum* resistance to antimalarial drugs, numerous studies have shown that mosquitoes are resistant to insecticides. In Gabon, the primary vector control strategies rely on long-lasting insecticidal nets and indoor residual spraying, with pyrethroids being the preferred insecticides due to their historical effectiveness. However, the emergence of insecticide resistance, reported in various regions of Africa and Asia [115], poses a significant threat to malaria control programs. In Gabon, the first reports of kdr-w and kdr-e mutations in

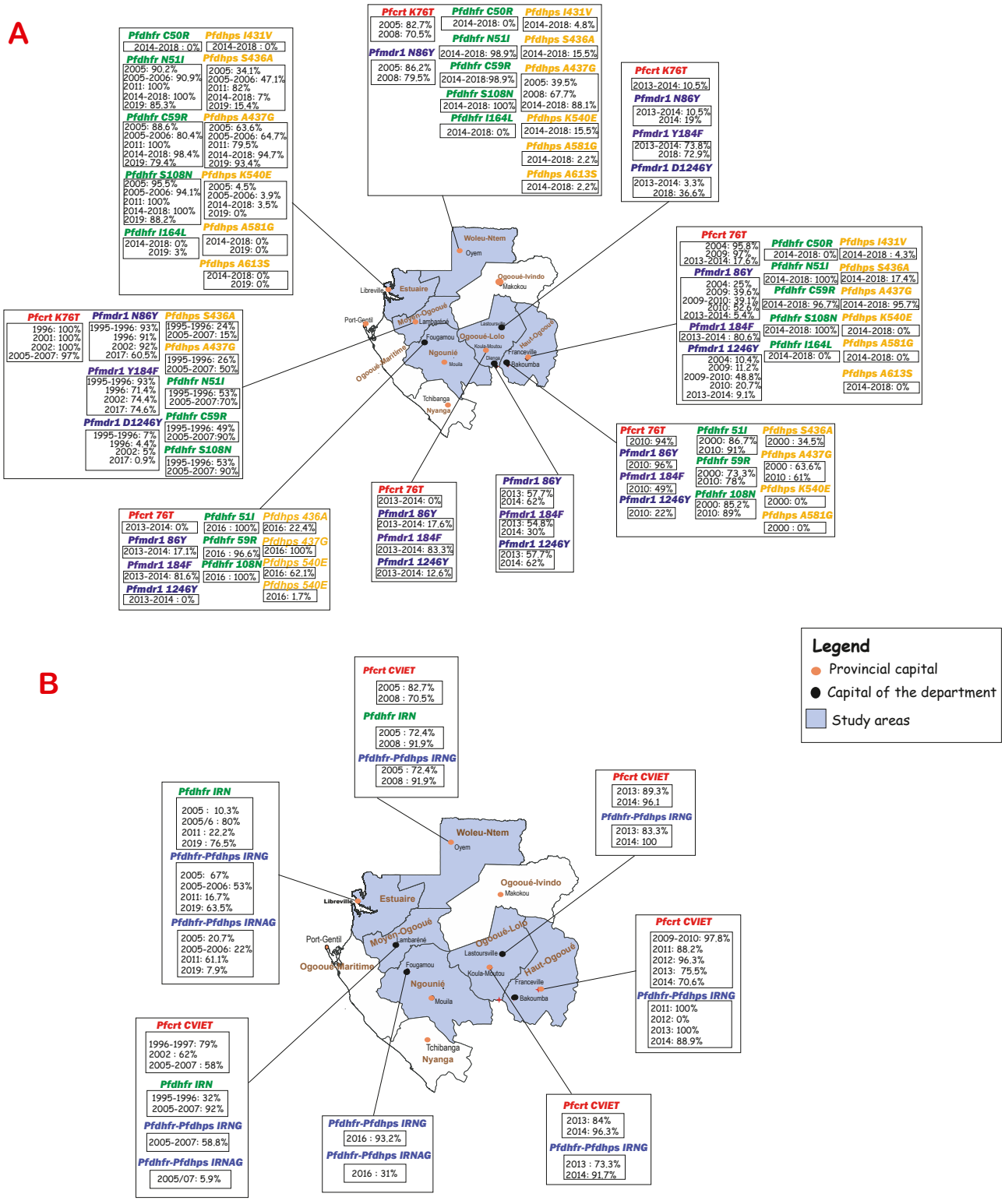

Fig. 4. Frequency and distribution of molecular larkers of antimalaria drug resistance in Gabon. 4 A: Spatial distribution of.

Anopheles mosquitoes emerged in 2000, with frequencies of 37 % and 63 %,respectively [58]. These mutations have spread to other regions of the country and their frequencies have been steadily increasing over time. These mutations have also been found in Cameroon[116], Equatorial Guinea [117] and Benin [23]. Contrary to what has been reported in certain West African countries such as Burkina Faso [22] and Senegal [118], no Ace 1 R mutations have been observed to date, apart from the one reported in 2006–2007 (0.4 %) [60]. In this respect, the use of organophosphates and carbamates as potential alternatives in vector control requires further study. However, the lack of data on the physiological and genetic characteristics of malaria vector resistance to conventional insecticides represents a significant gap in establishing an effective vector control system against malaria. This gap could be one of the underlying reasons for the stagnation in malaria control efforts in this heavily affected country such as Gabon.

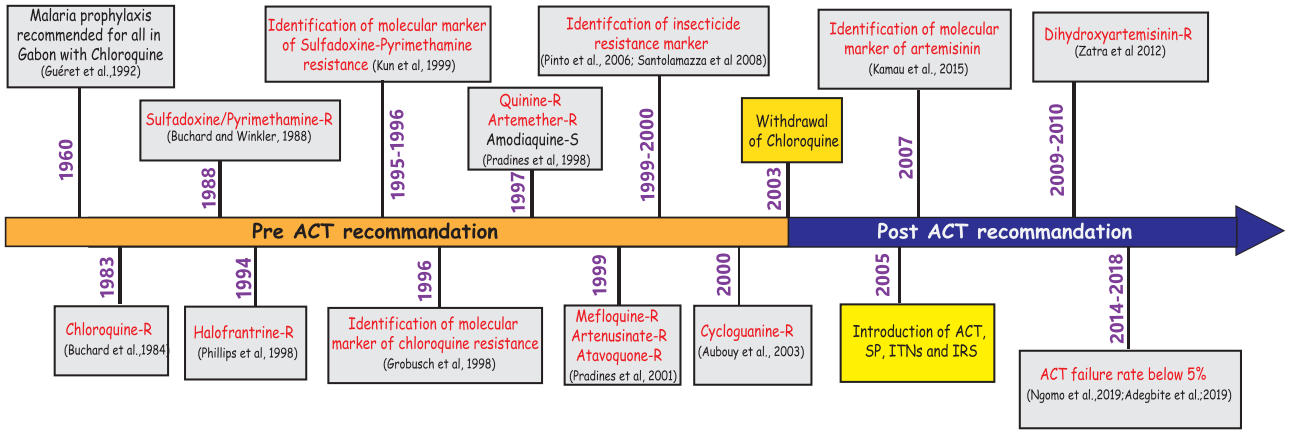

Fig. 5. Evolution of antimalaria drug resistance and insecticide resistance from 1980 to 2023 in Gabon.

Strengths and limitations of the review

The main strength of this review is that it addresses four major areas of study of malaria in Gabon, namely the epidemiology of infection, the efficacy of antimalarial treatments, the molecular epidemiology of antimalarial drug resistance, and vector resistance to insecticides. The present review provides a comprehensive overview of the prevalence and distribution of malaria, molecular markers of P. falciparum antimalarial drug resistance, and markers of insecticide resistance in both spatial and temporal terms in Gabon.

However, this study presents some limitations. First, there is a significant disparity in the amount of data available by temporal period and by province. Indeed, some provinces, especially in the south, southwest and west, appear to have been understudied for antimalarial drug resistance, vector resistance to insecticides and even malaria prevalence. Secondly, the limited number of samples or participants enrolled in some studies and the high heterogeneity across studies may not provide a true representation of the phenomena under study and may even affect the interpretation of the results. This high heterogeneity in the number of samples across studies led to the inclusion criteria for minimum sample size being less rigorous than they could have been. Furthermore, the malaria prevalence for children under five years of age was not analysed due to the limited number of studies providing prevalence results for this age group, which is the most vulnerable population group.

Conclusion

In Gabon, malaria is in recrudescence after a decline in prevalence following the implementation of WHO recommendations in the mid-2000s. This is due to non-compliance with WHO guidelines and the spread of *P. falciparum* resistance to antimalarial drugs and vector resistance to insecticides. Chloroquine resistance persists due to cross-resistance with other drugs in the same family. The use of SP in IPT for pregnant women has contributed to emergence genetic resistance to SP. However, IPT-SP remains effective, as evidenced by the low prevalence of malaria-associated factors in pregnant women. Although some failures have been reported following ACT treatment, their efficacy threshold remains above 90 %. At the same time, high levels of markers associated with vector resistance were identified. Overall, these data highlight the importance of further research into artemisinin resistance in *Plasmodium* and insecticide resistance in *Anopheles*, and, most importantly, the need to scale up this research across the country to improve malaria management at the national level.

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Declaration of Competing Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.jiph.2024.05.047.

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