UNIVERSITY OF CAPE COAST

GENETIC CHARACTERIZATION OF *PLASMODIUM* SPP AND PUTATIVE ANTIMALARIAL DRUG RESISTANT MARKERS IN THE CENTRAL REGION OF GHANA

MAVIS PUOPELLE DAKORAH

2023

UNIVERSITY OF CAPE COAST

GENETIC CHARACTERIZATION OF *PLASMODIUM* SPP AND PUTATIVE ANTI-MALARIA DRUG RESISTANT MARKERS IN THE CENTRAL REGION OF GHANA

BY

MAVIS PUOPELLE DAKORAH

A Thesis submitted to the Department of Biomedical Sciences of the School of Allied Health Sciences, College of Health and Allied Sciences, University of Cape Coast, in partial fulfilment of the requirements for the award of Doctor of Philosophy degree in Parasitology

FEBRUARY, 2023

DECLARATION

Candidate's Declaration

I hereby declare that this thesis is the result of my original research and that no part of it has been presented for another degree in this university or elsewhere.

Candidate's Signature: ..... Date: .....

Name: Mavis Puopelle Dakorah

Supervisors' Declaration

We hereby declare that the preparation and presentation of the thesis were supervised in accordance with the guidelines on supervision of the thesis laid down by the University of Cape Coast.

Principal Supervisor's Signature: ..... Date: .....

Name: Prof. Desmond Omane Acheampong

Co-Supervisor's Signature: ..... Date: .....

Name: Dr. Enoch Aninagyei

ABSTRACT

BACKGROUND: Malaria eradication is hampered by the genetic diversity of *Plasmodium falciparum*, antimalarial resistance, and eco-geographical distinctions. The Central Region of Ghana harbours different ecological zones which may drive genetic diversity and influence resistance patterns in malaria infections. **AIM:** To investigate the genetic characteristics of *Plasmodium* species and putative Antimalarial drug resistant markers in the Central region. **METHOD:** This was a cross sectional study involving 3993 samples collected during the dry and wet seasons from selected health facilities within the Forest and Coastal zones of the Central region. Bivariate analysis determined the association of malaria with independent variables. Selective whole genome amplicon sequencing (sWGA) assay was used to characterise 522 malaria-positive samples. **RESULTS:** Malaria prevalence was 61.8% by rapid diagnostic test and 61.1% by microscopy. Infection were associated with ecological zones ($p<0.001$) and seasons ($p<0.001$). Genomic analysis showed evidence of mixed *P. falciparum, P. vivax, and P. malariae* -(0.4%) amidst a high *P. falciparum* (98.9%) prevalence. Infections were mostly polyclonal (55.5%), ranging from one to six clones. Resistant haplotypes recorded were *Pfcrt* CVIET (0.5%), *Pfmdr1* NFD (44%), *Pfdhfr* triple mutation (N51I, C59R, S108N)-76.6%, *Pfdhps* SGKAA (32.8%), and *Pfdhps* SGEAA (0.3%), and *Pfkelch13* gene (48%).

CONCLUSION: Malaria was prevalent in all districts and influenced by eco-geographic factors among others. Resistance was observed in all antimalarials studied. Multifaceted yet targeted interventions are required all year round for effective malaria control.

ACKNOWLEDGMENTS

The All-Powerful God is the source of my deepest gratitude for His unfailing grace, love, mercies, and blessings. I wish to sincerely thank my supervisors, Prof. Desmond Omane Acheampong and Dr. Enoch Aninagyei for the privilege to study under you. Your enthusiasm and positivity are infectious and I do accept the challenge to propagate them to the best of my ability. Additionally, I am indebted to the Malaria Genome Laboratory at the Wellcome Sanger Institute in Hinxton, UK, which provided funding for the study's genomics component.

I am grateful to all lecturers in the Department of Biomedical Science for taking the time to critique and shape my work. Thank you, Juliana Attoh, for your friendship. I am also grateful to the University of Cape Coast for providing me with the ideal academic environment for my Ph.D. programme.

I am extremely thankful for the efforts of the management, staff, and patients of the St. Francis Xavier Hospital, Abura Dunkwa District Hospital, Agona Swedru municipal Hospital, Ewim Polyclinic, and the Saint Gregory Catholic Hospital. I am extremely thankful to the field teams led by Mr. Francis Gyebi, Mr. Sampson Gyabah, Mr. Jerry Kwame, Mr. Michael Nkansah, Mr. Manfred Ansah Nyame, Mr. Bertrand Asamoah Ackom, and Mr. Isaac Tukwarlba. I greatly value your assistance in this journey. You are the unsung heroes of my journey.

Thank you to my partner Godwin, my Parents Dr. and Mrs. Dakorah, and my siblings Dr. J.S. Dakorah and Mrs. L. B. Darko (Esq). I am truly appreciative of your constant support for me in everything I do. You make me bear my name with pride to call myself a Dakorah.

DEDICATION

I dedicate this work to my Mother Mrs. Grace Rosina Dakorah. To my husband Mr. Godwin Adzakpah, my children Nadine and Michael Adzakpah, and all study participants.

TABLE OF CONTENTS

	Page
DECLARATION	ii
ABSTRACT	iii
ACKNOWLEDGMENTS	iv
DEDICATION	v
TABLE OF CONTENTS	vi
LIST OF TABLES	xv
LIST OF FIGURES	xx
LIST OF ABBREVIATIONS	xxii
CHAPTER ONE: INTRODUCTION	
Background to the Study	1
Statement of the Problem	10
Purpose of the Study	11
Research Objectives	11
Research Questions	12
Rationale of the study	12
Delimitation of the Study	14
Limitation of the Study	14
Definition of Terms	15
Organisation of the Study	15
CHAPTER TWO: LITERATURE REVIEW	
Background	17
Malaria burden	17
The burden of Malaria in Ghana	19

Age distribution of malaria 22

Ecology, climatic factors and malaria transmission in Ghana 23

The life cycle of the malaria parasites 24

Signs and symptoms of malaria in human 27

The distribution of *Plasmodium* parasites 28

Gender distribution of malaria 28

Strategies for malaria control 30

Malaria diagnosis 30

Malaria control in Ghana 31

Molecular genotyping 32

Selective whole genome amplicon (sWGA) 33

Molecular Diagnosis in Ghana 34

Genetic diversity in *P. falciparum* 36

Complexity of infection 37

The complexity of infection in Africa 39

The complexity of infection in Ghana 39

Impact of ecological zones and climatic factors on Genetic diversity of *P. falciparum* 41

Genetic diversity of *P. falciparum* in ecological zones 41

Genetic diversity of *P. falciparum* and climatic factors 43

Genetic variation associated with antimalarial resistance 44

Polymorphisms associated with CQ resistance 48

Polymorphisms associated with CQ resistance in Ghana 49

Polymorphisms associated with Pyrimethamine 50

Polymorphisms associated with Sulfadoxine 52

Polymorphisms associated with Sulfadoxine–Pyrimethamine combination 54

Polymorphisms associated with SP resistance 57

Polymorphisms associated with Artemisinin (ACT) resistance 58

Artemisinin resistance-associated polymorphisms in Ghana 62

Polymorphisms associated with the *Pfmdr1* gene 63

Polymorphisms associated with partner drugs in Ghana (re look at it-contains non-Ghana articles) 66

Markers associated with Piperaquine resistance (Exonuclease gene) 66

Molecular studies on antimalarial resistance in Ghana 67

Conclusion 67

CHAPTER THREE: METHODOLOGY

Design and conceptual framework of the study 69

Ethical clearance and participants' informed consent 70

The study region 70

Ecological features of the region 71

Districts located in the Central Region area 71

Selection of study districts 71

Selection of study health facilities within study districts 72

Sample size calculation 74

Study population 74

Conditions for participant inclusion 75

Conditions for participant exclusion 75

Sensitization of health professionals regarding the study and training of research assistants 75

Participant enrolment 76

Informed consent 76

Criteria for withdrawal from the study 76

Procedures for sample collection 76

Collecting blood samples from adults 76

Collecting blood samples from children 77

Safety Considerations employed for sample collection: 77

Collection of study information 78

Sample transportation 78

Laboratory procedures 78

Malaria rapid diagnostic testing 79

Malaria microscopy and parasite counting 80

Quality Control 80

Criteria for selecting blood samples for preparation of dried blood spots 81

Preparation of DBS and associated metadata 82

Packaging and shipping of DBS 83

Genomic DNA extraction from DBS 84

Extraction of *Plasmodium* genomic DNA (gDNA) using Automated DNA extraction 84

.Selective whole genome amplicon sequencing (sWGA) assay 86

Multi-deplexing of sequences 87

Objective 2 89

Determination of COI 89

Objective 3 90

Data handling, statistical analysis, and analysis of genomic data 90

Analysis of baseline malaria data 90

Analysis of genomic data 91

CHAPTER FOUR: RESULTS AND DISCUSSION

Results 94

Objective 1 94

Total patient enrolees and distribution by district, seasons, and ecological zones 94

Demographic characteristics and parasitological outcome of the participants suspected of malaria 95

District and gender stratification of study participants 97

Age stratification of the study enrollees 97

Distribution of study participants by type of service sought 98

Prevalence of malaria by study district 99

Participants with malaria in the study sites stratified by age and patient type 100

Gender distribution of participants with malaria in the study sites 101

Distribution of participants with malaria according to patient type and study districts 102

Comparison of Malaria incidence in ecological zones and seasons 103

Simple logistic regression analysis of Malaria in the districts stratified by season and gender 104

Relationship between malaria and age stratified by district 106

Relationship between malaria and age stratified by ecological zone 107

Simple logistics regression of Malaria incidence and age stratified by season 108

Relationship between malaria and age stratified by gender 109

Malaria-associated risk factors among participants in the Central Region of Ghana 109

Objective 2 112

Molecular determination of the malaria parasites 112

Plasmodium species distribution in study districts 112

Distribution of *Plasmodium* species by ecology and gender 115

Distribution of species by seasons and gender 115

Sequencing success and failure rates 116

Distribution of clones by district 117

Variations of Mean COI within study districts 118

Overall mean Complexity of Infection (COI) by age group in all study sites 119

Distribution of COI by district and gender 120

Distribution of clones by ecological zones 121

Distribution of clones by ecology and seasons 123

Mean parasite density and complexity of infection 123

Objective 3 124

Antimalarial drug resistance markers in *P. falciparum* 124

Markers associated with resistance in the *Pfcrt gene* 126

Distribution of *Pfcrt haplotypes* by age-groups 128

Distribution of *Pfcrt haplotypes* by gender 130

Distribution of *Pfcrt* haplotypes by ecological zone 130

Distribution of *Pfcrt* haplotypes by seasons 131

Markers associated with resistance to *the Pfdhfr* gene 132

Distribution of *Pfdhfr* haplotypes by age-groups 135

Distribution of *Pfdhfr* haplotypes by gender 138

Distribution of *Pfdhfr* haplotypes by ecological zone 138

Distribution of *Pfdhfr* haplotypes by seasons 139

Markers of Resistance associated with *Pfdhps* gene 140

Distribution of wild and polyclonal *Pfdhps* haplotypes by districts 144

Distribution of *Pfdhps* monoclonal haplotypes stratified by age groups 147

Prevalence of wild and polyclonal mutant *Pfdhps* haplotypes by age-group 150

Distribution of *Pfdhps* haplotypes by gender 153

Prevalence of wild and mutant *Pfdhps* polyclonal haplotypes by gender 154

Distribution of *Pfdhps* haplotypes by ecological zone 155

Distribution of wild-type and polyclonal *Pfdhps* haplotypes by Ecological zone 157

Distribution of *Pfdhps* monoclonal haplotypes by seasons 158

Distribution of *Pfdhps* polyclonal haplotypes by seasons 159

Markers associated with Artemisinin resistance 160

Distribution of wild-type and mutant *Pfkelch13* Genes by district 160

Distribution of wild and multiple *Pfkelch13* mutant genes by district 164

Distribution of single mutant *Pfkelch13* gene by age-groups 167

Distribution of wild and multiple *Pfkelch13* mutations by age group 170

Distribution of *Pfkelch13* gene by gender 173

Distribution of wild and multiple *Pfkelch13* mutations by gender 174

Frequencies of wild and single mutant *Pfkelch13* haplotypes by the ecological zones 176

Distribution of wild and multiple *Pfkelch13* mutations by ecology 178

Distribution of *Pfkelch13* genes by seasons 179

Distribution of wild and multiple *Pfkelch13* mutations by Seasons 181

Markers associated with resistance to ACT partner drugs (Amodiaquine and Lumefantrine) in the *Pfmdr1* gene 182

Distribution of *Pfmdr1* genes by patient age groups 183

Distribution of *Pfmdr1* haplotypes by gender 186

Distribution of *Pfmdr1* haplotypes by ecological zone 187

Distribution of *Pfmdr1* by seasons 188

Markers associated with exonuclease gene 189

OBJECTIVE 4: Prevalence of haplotypes associated with *P. Falciparum* antimalarial drug resistance in the study districts 189

Determinants of *Pfkelch13* resistance 190

Exploring factors influencing putative drug-resistant haplotype to *Pfcrt* gene 192

Exploring factors influencing Putative Pyrimethamine Resistant Haplotype in *Pfdhfr* gene 194

Exploring factors influencing putative Sulfadoxine resistant Haplotype in *Pfdhps* 196

Exploring factors influencing Putative Drug Resistant Haplotype in *Pfmdr 1* gene 198

Exploring factors influencing Putative Drug Resistant Haplotype to *Pfdhfr/Pfdhps* mutations 201

Discussion 203

CHAPTER FIVE: SUMMARY, NOVELTY, CONCLUSIONS AND RECOMMENDATION

Summary 245

Novelty 247

Conclusions 248

Recommendations 249

REFERENCES 252

APPENDICES 312

LIST OF TABLES

Table		Page
1	Major SNPs associated with antimalarial drug resistance	45
2	The selected districts and their population for 2010	74
3	Distribution of participants by district, seasons, and ecological zones	95
4	Demographic characteristics of participants	96
5	Gender prevalence of the study participants	97
6	Age distribution of the study participants	98
7	Percentage distribution of Malaria by RDT and Microscopy	100
8	Ecological and Seasonal distribution of malaria cases	104
9	Simple logistic regression analysis of Malaria in the districts stratified by season and gender	105
10	Simple logistics regression of Malaria incidence and age stratified by Assin Central Municipality and Gomoa East District	106
11	Simple logistics regression of Malaria incidence and age stratified by Abura-Asebu-Kwamankese District and Cape Coast Metropolis	107
12	Simple logistics regression of Malaria incidence and age stratified by Agona Swedru Municipality	107
13	Simple logistics regression of Malaria incidence and age stratified by Ecological zone	108
14	Simple logistics regression of Malaria incidence and Age Stratified by Season	109
15	Simple logistics regression of Malaria incidence and age stratified by gender	109

16	Unadjusted and Adjusted Logistic Regression of risk factors of Malaria prevalence among participants in the Central Region of Ghana	111
17	Distribution of Species in Study District by Gender and Ecology	114
18	Distribution of Species by Ecology and Gender	115
19	Distribution of Species by Seasons and Gender	116
20	Relationship between COI and Study District	118
21	Variations of Mean COI within study districts	119
22	Overall Mean COI by Age-group in all study sites	119
23	Distribution of Clones by District and gender	121
24	Distribution and Relationship among Clones and Gender stratified by Ecological zones	122
25	Mean Clones by Ecological zones and seasons	123
26	Mean parasite density by clones	124
27	Distribution of successfully sequenced genes by districts	125
28	Distribution of wild and mutant *Pfcrt* haplotypes by districts	127
29	Prevalence of wild and mutant *Pfcrt* haplotypes stratified by patient age	129
30	Prevalence of wild and mutant *Pfcrt* haplotypes by gender	130
31	Distribution of *Pfcrt* haplotypes by Ecological zone	131
32	Distribution of *Pfcrt* haplotypes by Seasons	132
33	Distribution of wild and mutant *Pfdhfr* haplotypes by districts	134
34	Prevalence of wild and mutant *Pfdhfr* haplotypes by Age group	136
35	Prevalence of wild and mutant *Pfdhfr* haplotypes by gender	138
36	Distribution of *Pfdhfr* haplotypes by ecological zone	139

37	Distribution of *Pfdhfr* haplotypes by Seasons	140
38	Distribution of wild and mutant *Pfdhps* gene by districts	142
39	Distribution of wild and polyclonal *Pfdhps* haplotypes by districts	145
40	Prevalence of wild and mutant *Pfdhps* monoclonal haplotypes by age group	148
41	Prevalence of wild and polyclonal mutant *Pfdhps* haplotypes by age group	151
42	Prevalence of wild and mutant *Pfdhps* haplotypes by gender	153
43	Prevalence of wild and mutant *Pfdhps* polyclonal haplotypes by gender	155
44	Distribution of *Pfdhps* haplotypes by ecological zone	156
45	Distribution of wild-type and polyclonal *Pfdhps* haplotypes by Ecological zone	158
46	Distribution of *Pfdhps* monoclonal haplotypes by seasons	159
47	Distribution of wild type and polyclonal *Pfdhps* haplotypes by Seasons	160
48	Distribution of wild and single mutant *Pfkelch13* gene by district	162
49	Distribution of wild and multiple *Pfkelch13* mutations by district	165
50	Prevalence of wild and single mutant *Pfkelch13* gene by Age group	168
51	Distribution of wild and multiple *Pfkelch13* mutants by Age group	171
52	Prevalence of wild and single mutant *Pfkelch13* by gender	174
53	Prevalence of wild and multiple mutants *Pfkelch13* gene by gender	175
54	Distribution of single and mutant *Pfkelch13* gene by Ecological zone	177

55	Distribution of wild and multiple *Pfkelch13* mutations by Ecological zones	179
56	Distribution of *Pfkelch13* wild-type and single mutations by Seasons	180
57	Distribution of *Pfkelch13* wild-type and multiple mutants by Seasons	181
58	Distribution of wild and mutant *Pfmdr1* haplotypes by districts	183
59	Prevalence of wild and mutant *Pfmdr1* haplotypes by Age group	185
60	Prevalence of wild and mutant *Pfmdr1* haplotypes by gender	186
61	Distribution of *Pfmdr1* haplotypes by Ecological zone	187
62	Table Distribution of *Pfmdr1* haplotypes by Seasons	188
63	Distribution of wild *Pf-exonuclease* genes by districts	189
64	Haplotypes of mutations of epidemiological importance	190
65	Unadjusted and Adjusted Logistic Regression of factors influencing *Pfkelch13* resistance in *Pfkelch13* among participants in the Central Region of Ghana	192
66	Unadjusted and Adjusted Logistic Regression of factors influencing putative drug resistance haplotypes to Chloroquine among participants in the Central Region of Ghana	194
67	Unadjusted and Adjusted Logistic Regression of factors influencing Putative Drug Resistant Haplotype to *Pfdhfr* gene	196
68	Unadjusted and Adjusted Logistic Regression of factors influencing Putative Sulfadoxine Resistant Haplotype to *Pfdhps*	198

69	Unadjusted and Adjusted Logistic Regression of factors influencing Putative Drug Resistant Haplotype in *Pfmdr* 1 gene among participants in the Central Region of Ghana	200
70	Unadjusted and Adjusted Logistic Regression of factors influencing putative drug resistance haplotypes to SP among participants in the Central Region of Ghana	202
71	Distribution of demographic and clinical variables of the malaria by five districts in the Central Region	313

LIST OF FIGURES

Figure		Page
1	Distribution of Malaria Cases Worldwide (WHO, 2020).	2
2	The distribution of malaria prevalence at survey locations (left panel) and regional estimates (right panel) in Ghana are depicted on a map of the country. Adopted from: (Ejigu & Wencheko, 2021)	21
3	Ghana map showing the regions within each malaria epidemiological zone	24
4	Sexual and Asexual life cycle of *P. falciparum*	27
5	Diagram showing the complexity of infection	37
6	The study's conceptual framework	70
7	Map of Central Region indicting selected study districts in the forest (Green) and coastal (Orange) zones and other districts (Blue)	73
8	Conceptual Framework-Showing the pattern of workflow employed in the study	79
9	Giemsa stained blood film	81
10	An unstained blood film	81
11	*P. falciparum* trophozoites in the stained thin blood film	81
12	DBS preparation kit	82
13	Pictorial description of the preparation of dry blood spots	83
14	Pictorial description of preparations per sample labelled with barcodes	83
15	Packaging of DBS before shipping	84

16	Direction of flow of gDNA extraction and quantification	86
17	Shows bioinformatic pipeline for the analysis of Sequenced *Plasmodium* genes.	88
18	Distribution of patient types in the health facilities. There is a statistically significant association between study districts and patient types in the health facilities	99
19	Distribution of participants with malaria according to age ranges and study districts	101
20	Distribution of participants with malaria according to gender and study districts	102
21	Distribution of participants with malaria according to patient type and study districts	103
22	Map of study sites showing amplification success and failure	112
23	Map of study sites showing Sequencing success and failure	117
24	Map of Central Region showing gene prevalence per study site	126
25	Prevalence of cumulative *Pfkelch13* gene by gender and district	176

LIST OF ABBREVIATIONS

ACTs	-	Artemisinin Combination Therapies
AL	-	Artemether Lumefantrine
AMP-Seq	-	Amplicon Sequencing
ASAQ	-	Artesunate + Amodiaquine
PQ	-	Piperaquine
SP	-	Sulfadoxine-pyrimethamine
CQ(R)	-	Chloroquine (Resistance)
DHA-PP	-	Dihydroartemisinin + Piperaquine
*Pf*dhfr	-	*P. falciparum* dihydrofolate reductase
*Pf*dhps	-	*P. falciparum* dihydropteroate Synthase
Pfcrt	-	*P. falciparum* Chloroquine Resistance Transporter
*Pf*mdr-1	-	*P. falciparum* Multidrug Resistance 1
NGS	-	Next Generation Sequencing
PCR	-	Polymerase Chain Reaction
WT	-	Wild-type
SNPs	-	Single Nucleotide Polymorphisms
CI	-	Confidence Interval
DBS	-	Dried Blood Spots
DNA	-	Deoxyribonucleic Acid
NMCP	-	National Malaria Control Programme
PMI	-	President's Malaria Initiative
RDTs	-	Rapid diagnostic tests
WHO	-	World Health Organization

CHAPTER ONE

INTRODUCTION

Background to the Study

Malaria is a common infection even though lethal in nearly 40% of the world's population. Despite concerted eradication efforts, the availability of affordable and effective chemotherapy, and multiple public health interventions (WHO, 2016; WHO 2018a), the disease still persist. From 2000 to .2015, the estimated number of cases and deaths from malaria decreased by 27%, from 238 million cases and 736,000 deaths in 2000 to 229 million cases and 409,000 deaths in 2019 (WHO, 2020). These drop in malaria cases and deaths were due to malaria control efforts according to the World Health Organisation (WHO). The infection is no longer a burden in Europe, North America, and Russia (Nájera, González-Silva, & Alonso, 2011) with China being the most recent to rid itself of malaria (Chen, Fen, & Zhou, 2021). However, malaria endemicity remains high in tropical regions of Africa where an estimated 92% of infections and 93% of deaths are reported to occur (WHO, 2020; WHO, 2018) and threatens the life of children less than five years and expectant mothers (Ippolito, Moser, Kabuya, Cunningham, & Juliano, 2021).

Ghana has benefited from many malaria control interventions yet the burden and severity of the infection are still high in children and expectant mothers (Afutu, Boampong, & Quashie, 2021). In 2017, 40% of outpatients were infected with malaria (Awine, Malm, Bart-Plange, & Silal, 2017) with 10.2 million suspected malaria infections according to the National Malaria Control Programme (NMCP, 2017). Two years after, Ghana was named among the 10 high malaria -burdened African countries (WHO, 2019),

registering the greatest increase in cases of malaria in 2018 (Ofori et al., 2021) and contributing about 3% to the global burden (WHO, 2019). The global distribution of malaria cases is depicted in Figure 1.

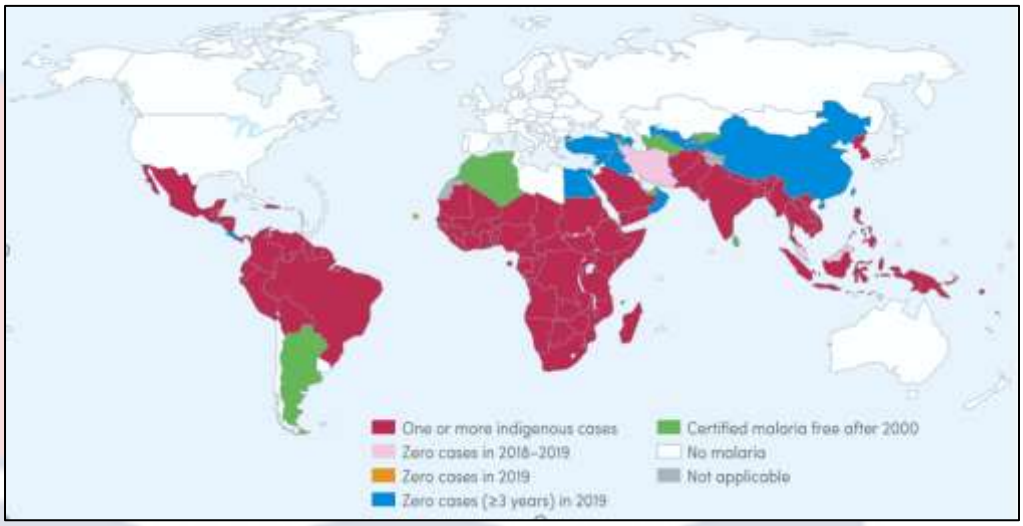


Figure 1: Distribution of Malaria Cases Worldwide (WHO, 2020).

The female Anopheles mosquito serves as a vector for five *Plasmodium* species that currently infect humans. These are *Plasmodium falciparum, Plasmodium ovale, Plasmodium vivax, Plasmodium malariae,* and *Plasmodium knowlesi* (Zhang, Xie, Xie, & Bourne, 2016) . *P. falciparum* and *P. vivax* are the most common worldwide. On the other hand, *P. falciparum* is the most virulent species frequently detected in sub-Saharan Africa (SSA) (Ashley, Pyae Phyo, & Woodrow, 2018; Ippolito et al., 2021; WHO, 2017b) and in 95.0% of infections in Ghana (Amoah et al., 2019; Awine et al., 2017; Njiro et al., 2022). Low frequencies of *P. malariae* and *P. ovale* are usually recorded as mixed infections with *P. falciparum*. However, a recent study in the Kwahu South districts of the Eastern Region of Ghana reported a prevalence of 12.7% for *P. malariae* (Owusu, Brown, Grobusch, & Mens, 2017) whilst *P. vivax* is yet to be reported (Awine et al., 2017). Also, a high prevalence of *P. falciparum* has been recorded in neighbouring Togo

(Kombate et al., 2022), La Cote d'Ivoire (Silué et al., 2021), Nigeria (Awosolu, Yahaya, Haziqah, Simon-Oke, & Fakunle, 2021) and Burkina Faso (Grant et al., 2022). The dominance of *P. falciparum* is also reported in Gabon (Boukoumba et al., 2021), Mozambique (Chidimatembue et al., 2021), and Ethiopia (Almaw, Yimer, Alemu, & Tegegne, 2022).

The WHO's global technical strategy for malaria control focused primarily on prompt, effective treatment and early diagnosis (hypnozoites excluded) (Roux et al., 2021; WHO, 2016). Diagnosis of malaria, in resource-limited settings including Ghana, relies heavily on microscopy (gold standard) which requires visualization of the causative parasites. However, its use is hindered by the limited number of skilled microscopists which could result in difficulty in species differentiation, misdiagnosis as a result of low parasitaemia or mixed infections (Benié et al., 2022). More recently rapid diagnostic tests (RDTs), have been instituted. This test looks for the histidine-rich protein II (HRP2) of the parasite, as well as the parasite's lactate dehydrogenase (pLDH) or p-aldolase (p-aldolase) during the erythrocytic cycle (Fitri et al., 2022; Ugah et al., 2017). Nevertheless, RDTs are constrained with false positives due to the persistence of HRP2 in the blood and false negatives due to gene deletions (Fitri et al., 2022; Kozycki et al., 2017). Additionally, fluorescent microscopy, serology, isothermal amplification, and polymerase chain reaction (PCR) can be used for the detection of malaria parasites in the blood (Aninagyei, Stella., Boye, Egyir-Yawson, & Acheampong, 2019; Benié et al., 2022; Fitri et al., 2022). However, for epidemiological studies and infection mapping, the WHO

suggests utilizing nucleic acid amplification tests (NAATs) (Fitri et al., 2022; Omedo, 2017).

Apart from the use of microscopy and RDT, most molecular epidemiological studies on malaria in Ghana have involved PCR- based techniques and microsatellite markers, however, these techniques allow for the study of only a small proportion of the genome which excludes the identification of SNPs and rare alleles in the section of the genome not studied (Osborne et al., 2021). This challenge is overcome by the use of next-generation sequencing (NGS) technologies like selective whole genome amplicon sequencing (sWGA). The sWGA, employs the phi polymerase to selectively amplify the target *Plasmodium* DNA with specific primer sets in a pre-amplification step. This allows simultaneous differentiation and amplification of DNA from different parasites (Aninagyei et al., 2019; Leichty & Brisson, 2014; Oyola et al., 2016)

A low mortality of roughly 0.1% is attainable with effective antimalarial chemotherapy in uncomplicated malaria (Ashley et al., 2018). Malaria chemotherapy particularly for *P. falciparum* has evolved rapidly compared to that in non-falciparum species. This is mostly attributed to parasite biology, as the formation of hypnozoites in the liver helps *P. vivax* and *P. ovale* to evade blood schizonticides) (Molina-Cruz, Zilversmit, Neafsey, Hartl, & Barillas-Mury, 2016). Several medications, mostly in monotherapy were used for malaria prevention. Notwithstanding, the reduction in the efficacy of antimalarial monotherapy caused a resurgence of malaria infections with increased morbidity and mortality. As a result, the World Health Organization (WHO) approved Artemisinin combination

therapies (ACTs) as the first-line treatment for uncomplicated *P. falciparum* malaria infections (Sayang et al., 2009). ACT has been cited as a reason for the significant reduction in malaria-related morbidity and mortality experienced from 2002-2015 (Amaratunga et al., 2016). The principle behind its efficacy is a rapid parasite clearance time of artemisinin derivatives (Artemether, Artesunate, or Dihydroartemisinin) combined with less potent but longer-acting agents (Lumefantrine, Amodiaquine, Mefloquine, Piperaquine, Sulphadoxine-pyrimethamine or Pyronaridine) (Greenwood, 2017). Nonetheless, the mechanism of action, promotes resistance to the partner agents while slowing the progression of artemisinin resistance (Ippolito et al., 2021). ACTs are noted for their effectiveness against both falciparum and non-falciparum malaria parasites (Buyon, Elsworth, & Duraisingh, 2021).

Prior to the use of ACTs in Ghana, Chloroquine was the most used antimalarial. However, Chloroquine was replaced as the first-choice antimalarial chemotherapy by artemisinin-based combination therapy (ACT) due to widespread *P. falciparum* resistance (Mensah et al., 2020) . Amodiaquine/Sulfadoxine-pyrimethamine (AQ/SP) for seasonal malaria chemoprevention in children (IPTc) and Sulfadoxine-pyrimethamine (SP) for intermittent preventive treatment in pregnancy (IPTp), have also been recommended for these high-risk groups in Ghana (Ansah et al., 2021; MOH., 2014). Doxycycline, mefloquine, and atovaquone-proguanil (Malarone) are also suggested as chemoprophylaxis for travellers moving from non-endemic regions to endemic settings. Artesunate-amodiaquine (ASAQ), artemether-lumefantrine (AL), and dihydroartemisinin-piperaquine (DHAPQ) are the

ACTs that are currently recommended for use in Ghana (Asare et al., 2021). Over a decade after its implementation, ACTs remain effective in malaria control (Ofori et al., 2021).

P. falciparum infections in natural endemic settings are characterised by the co-existence of genetically distinct parasite clones in a susceptible human host, particularly in Africa where there is a high intensity of malaria transmission (Auburn & Barry, 2017; Aydemir et al., 2018; MalariaGEN, 2021; Zhong, Koepfli, Cui, & Yan, 2018). This is known as Clonal diversity. This phenomenon can increase the generation and spread of drug-resistant parasite clones and the likelihood of treatment failure (Dara et al., 2022). According to some research, there are more infections in high transmission areas where they recorded multiple *P. falciparum* clones and fewer clones in areas with low transmission (Amambua-Ngwa et al., 2019; Matrevi et al., 2022). Thus COI has been used to characterize malaria transmission intensity (Auburn & Barry, 2017; Gueye et al., 2018; Zhong et al., 2018) and evaluate public health interventions over time (Ariey et al., 2014; Neafsey, Taylor, & MacInnis, 2021). Additionally, the various species of the parasites exist at different frequencies in different geographic areas, influencing the prevalence of specific species, the distribution of parasites genotypes, and the allelic polymorphisms of Plasmodium in individuals presenting different complexities of infection. Recent studies demonstrate that distinct ecological factors can modulate the diversity of *P. falciparum* clones (Dara et al., 2022; Matrevi et al., 2022).

Transmission of malaria is widespread in Ghana, with seasonal peaks and varying endemicity along the country's diverse ecological zones (Amoah.,

et al., 2021; Duah, Matrevi, Quashie, Abuaku, & Koram, 2016). Thus, Characterizing *P. falciparum's* genetic diversity using measures of polyclonality will help define the distribution dynamics and genetic structure of the parasite population (Carlton et al., 2015; Fola et al., 2017; Mita & Jombart, 2015)

Genetic polymorphism in *Plasmodium* species particularly *P. falciparum* helps the parasite to undergo antigenic shift during its life cycle enabling the parasites to adjust to environmental changes, invade the host immune system and confer reduce sensitivity or resistance to drugs (Molina-Cruz et al., 2016). The ability of a parasite strain to survive and/or reproduce despite adequate administration of the recommended dose and absorption of an antimalarial drug in clinical use is known as antimalarial drug resistance (WHO, 2010). This characteristic of *P. falciparum* challenges malaria treatment efficacy. It has also resulted in treatment failure, the halt of control and elimination programmes, and the evolution in treatment policies observed across the world (Molina-Cruz et al., 2016; WHO, 2010). Antimalarial drug resistance can occur de novo (Yobi et al., 2022) or by specific drug pressure which may drive selection, adaptation, and population fragmentation, leading to the genetic diversity in the parasite population (Amambua-Ngwa et al., 2019; Mama et al., 2022). Such mutations may first arise independently in a region after which it invades other areas (Uwimana, 2020).

Single nucleotide polymorphisms (SNPs) in *P. falciparum* genes encoding a vacuolar membrane transporter protein and enzymes involved in folate synthesis were found to be the primary cause of antimalarial resistance after several studies (Breglio et al., 2018). Antimalarial drug resistance

markers include those for Quinine (*Pfcrt, Pfmdr1, Pfnhe1*), Sulfadoxine (*Pfdhps*), Pyrimethamine (*Pfdhfr*), Chloroquine and Amodiaquine (*Pfcrt, Pfmdr1*), Artemisinin (*Pfkelch13*), and Sulfadoxine (*Pfdhps*) (Breglio et al., 2018; Ippolito et al., 2021).

Resistance to Chloroquine and other quinoline- and artemisinin-based agents is linked to amino acid changes in the *P. falciparum* Chloroquine Resistance Transporter (*Pfcrt*) gene at codons 72, 74, 75, and 76, respectively. The CVIET haplotype ensures CQ resistance throughout Africa, whereas the SVMNT haplotype thrives in South America and Papua New Guinea (Yobi et al., 2022). *P. falciparum* multidrug resistance 1 (*Pfmdr1*) has an impact on the sensitivity of several antimalarials and is a member of the drug metabolite transporter (DMT) family. The *Pfmdr1* is a homolog of human P-glycoproteins with mutations linked to resistance at codon: N86Y, Y184F, D1246Y, S1034C, and N1042D (Aninagyei, Acheampong, Ampomah, Egyir-Yawson, & Boye, 2020; Aydemir et al., 2018; Buyon et al., 2021; Cheng, Htoo, Mhote, & Davison, 2021; Chidimatembue et al., 2021; Ebel, Reis, Petrov, & Beleza, 2021; Ippolito et al., 2021; Mensah et al., 2020). Generally, different codons in the *Pfmdr1* are selected to indicate resistance or susceptibility during antimalarial chemotherapy. *Pfmdr1* haplotype YYY (86Y, Y184, and 1246Y) and NFD haplotype (N86, 184F, and D1246) is associated with ASAQ and AL respectively. Similar mutations have been reported in Ghana, around the continent and in imported malaria cases from Africa to China (Aninagyei, Acheampong, Ampomah, Egyir-Yawson, & Boye, 2020; Aydemir et al., 2018; Cheng et al., 2021; Ebel, Reis, Petrov, & Beleza, 2021; Mensah et al., 2020) . Pyrimethamine (PYR) and Sulfadoxine

(SDX) are antimalarials that inhibit the enzymes dihydrofolate reductase (*dhfr*) and dihydropteroate synthase (*dhps*) in the redox cycle and the biosynthesis of folate. Haplotypes with varying degrees of drug resistance to sulphadoxine, pyrimethamine, or both contain multiple mutations in the individual gene or a combination of mutations in both *Pfdhps* and *Pfdhfr* (Mahamar et al., 2022). Mutations in DHPS (*Pfdhps*) gene, causes resistance in Sulfadoxine whilst that in DHFR (*Pfdhfr*) gene confer resistance to Pyrimethamine. Modified amino acid substitutions at codons *Pfdhfr*-C50R, *Pfdhfr*-N51I, *Pfdhfr*-C59R, and *Pfdhfr*-I164L are the root cause of resistance in *Pfdhfr*. Mutation S436A/F, A437G, K540E, A581G, and A613S mutations confer resistance to Sulfadoxine in *Pfdhps* (Amenga-Etego et al., 2021; Roux et al., 2021).

Artemisinin resistance has been linked by genetic analysis to polymorphisms in the *Pfkelch13* gene (Takala-Harrison et al., 2015) and the cause of the slow parasite clearance rates associated with ACT in western Cambodia, Thailand, Vietnam, and Myanmar (Ashley et al., 2014; Brown et al., 2015; Kumar et al., 2015; Li et al., 2015; Rouhani, Zakeri, Pirahmadi, Raeisi, & Djadid, 2015; Tun et al., 2015).

Mutations of P574L, F446I, I543T, R539T, and C580Y have been identified as the culprits in South East Asia. However, the primary mutation associated with ACT resistance is the propeller domain C580Y mutation (Ménard et al., 2016; Ocan et al., 2019). Recent reports of *Pfkelch 13* mutations have been made in Ghana with a C580Y mutation (Aninagyei, Acheampong, Ampomah, Egyir-Yawson, & Boye, 2020) and Rwanda with *Pfkelch13* R561H, P574L, and C469Y (Uwimana, 2020). Similarly, a recent

survey found variants of *Pfkelch13* mutations prevalent in Ghanaian *P. falciparum* parasites (Matrevi et al., 2019). These variants were of mutations responsible for artemisinin resistance in Asia and some African countries and may severely undermine malaria control efforts.

Of further concern is the report of Clinical dihydroartemisinin-piperaquine (DP) failure in Southeast Asia associated with increased *plasmepsin 2* copy number and an E415G encoding mutation in the exonuclease gene (Amato et al., 2017). In parts of Cambodia, Thailand, and Vietnam, the prevalence of DHA-PPQ treatment now exceeds 50% (van der Pluijm et al., 2019). Despite the rise and spread of ACT resistance throughout Southeast Asia (Ashley et al., 2014; WHO, 2015), the drugs continue to be the first-line treatment for uncomplicated malaria, with high efficacy and rapid clearance rates in sub-Saharan Africa (SSA) (Ashley et al., 2014).

The impact of genetic diversity on malaria control are limitless and very diverse in different settings (Recker, Bull, & Buckee, 2018). Thus, variations in local parasites and their impact on the epidemiological patterns of infection ought to be taken into account for effective control and elimination (Roca-Feltrer et al., 2010).

Statement of the Problem

The prevalence of malaria in Sub-Saharan Africa has remained unabated despite ongoing control efforts over the past five decades. Interventions such: as the Roll Back Malaria initiative, vector control (ITN and IRS), and change in drug policy from monotherapy to combination therapy (Bhatt et al., 2015; WHO, 2010), are major efforts against malaria these past decades. The persistence of malaria may be due to genetic variation

in the parasite population, a characteristic, not readily available through routine diagnostic testing. An epidemiologic study of the parasite genome will thus help to better understand malaria transmission and evaluate public health interventions. Furthermore, data on the heterogeneity of parasites in Ghana is finite. Before drug-resistance mutations jeopardize the effectiveness of current malaria treatments, it is essential to investigate the genetic diversity of malaria parasites in various ecological settings across the nation. Such exploits are the bedrock of novel strategies for the elimination of malaria parasites.

The current research focuses on *P. falciparum*'s molecular epidemiology in two ecological settings in Ghana's Central Region.

Purpose of the Study

The study aims to assess the molecular epidemiology of *P. falciparum* malaria infections in the central region of Ghana.

Research Objectives

The study's specific objectives are to:

1. Investigate the prevalence and epidemiological risk factors of malaria in multi-sites in the Central Region of Ghana.
2. Evaluate and compare the *P. falciparum* population structure and intensity of malaria transmission by describing the clonal complexity of infections in two distinct ecological zones (forest and coastal zones) during seasonal patterns in the Central Region.
3. Assess parasite gene mutation frequencies in *Pfcrt, Pfdhfr*, *Pfdhps, Pfmdr1, Pfexonuclease,* and *Pfkelch13* genes in the Central Region.

4. Investigate *Plasmodium* genetic mutations that confer resistance to various anti-malaria drugs and model the association between putative drug resistance haplotypes and independent variables.

Research Questions

1. What is the malaria prevalence and risk factors among participants in the Central Region?
2. What is the parasite population structure and do the ecological zones within the Central Region have clonal complexity of infections (COI) within the seasons?
3. Are there single nucleotide polymorphisms at various antigenic loci that can lead to drug resistance (*Pfcrt*, *Pfdhfr*, *Pfdhps*, *Pfmdr1*, *Pfexonuclease, Pfkelch13* genes)?
4. What are the risk factors of *P. falciparum* antimalarial drug resistance haplotypes in the study districts?

Rationale of the study

Malaria burdens the Central Region of Ghana which houses two ecological zones (a coastal savannah and a tropical rain forest). According to the 2016 malaria indicator survey, 30.2% and 44.6% of children aged between 6-59 months in the central region, tested positive for malaria by microscopy and RDT respectively. On the other hand, in 2019, 29.9% RDT-positive cases and 17.6% microscopy were recorded after testing 186 children in 2019 (GSS, 2017, 2021). This provides sufficient evidence that malaria control efforts at the individual-level such as bed net distributions, antimalarial treatments, and indoor residual spraying have not been able to significantly reduce malaria cases in the Central region.

The relationship between genetic diversity and clinical outcome is evident in several studies in the Eastern, Western, Ashanti, and Greater Accra regions of Ghana. These have revealed variations in transmission intensity and alterations in the genetic landscape of parasites within ecological zones in separate regions. However, no study has compared these parameters within the same region with diverse ecological settings. Furthermore, current data on local micro eco-climatic factors suggests that the genetic population structure of the parasites in these two ecological zones as found in the central region may differ. However, assessing the influence of ecological determinants on the local transmission intensity and parasite genetic profile, has been least explored in the central region compared to the readily available data on other regions of the country.

Understanding the local dynamics of *P. falciparum* genotypes is needed for developing control strategies in the region. Thus, this study bridges the knowledge gap on the current molecular epidemiology of *P. falciparum* parasites within the Central region of Ghana. Nonetheless, monitoring of genetic diversity and COI in the Central region is in line with the WHO recommendation to conduct therapeutic efficacy studies at least once every 24 months to allow early detection of changes in antimalarial efficacy (GSS, 2017). This study will help to identify a vulnerable population that requires immediate attention (e.g., certain age groups). This study will reveal the impact of malaria control programmes on genomic variation and its impact on present antimalarial chemotherapy as well as the population structure in similar or distinct eco-geographical settings of the region. It will possibly lead to the identification of novel loci of clinical relevance to malaria control.

Delimitation of the Study

Due to the broad nature of genetic diversity in the *Plasmodium* parasite, this study focused on two key elements: the complexity of infection and drug resistance in the *Plasmodium* parasite. Also, five districts out of twenty-two districts in the Central Region were studied amidst its ecogeographic diversity and malaria endemicity. Thus, the molecular epidemiological patterns presented may not be representative of the entire Central Region. However, the findings of the observed mutations provide baseline information for surveillance. Community-based Health Planning and Services (CHPS) compounds were excluded from the list of health facilities in selecting the study facilities due to the unavailability of malaria microscopy services in these health facilities.

Limitation of the Study

This study was hospital-based with sample collection beginning in 2020, during the COVID-19 pandemic. This could have affected the health seeking behaviour of persons living in the districts and underestimated the incidence of variables studied. Out of the 522 samples amplified, successfully sequenced genes ranged from 358 (87.5%) to 403 (100%). The presence of other blood-borne parasites such as *Babesia* species undifferentiated by microscopic examination could have resulted in reduced parasite amplification. Similarly, this could have resulted from low parasite density. Nonetheless, sequencing failure could influence the prevalence of SNPs as well as haplotypes presented. This may be due to low parasite density leading to low DNA yield. Also, the *Pfmdr1* and *Pfplasmepsin2* copy numbers measurements, for mefloquine and Piperaquine resistance were not carried

out. The study period may have been insufficient to detect any pattern of selection. Thus, differences in genetic diversity and COI in the two ecological zones are temporal. Other potential risk factors of malaria such as humidity and temperature were not estimated alongside climatic factors for a holistic assessment of critical drivers.

Definition of Terms

The Complexity of infection (COI): is the mean number of parasite genotypes of *P. falciparum* natural infections per individual.

Polyclonal infection: An infection caused by genetically distinct clones of *P. falciparum* clones in a susceptible host.

Clonal infections/ *P. falciparum* monoclonal infection: Clonal infections occur when an individual is infected with only one parasite clone of *P. falciparum*.

Molecular markers: these are a fragment of DNA sequences associated with a genome and are used to identify a particular sequence.

Malaria control: is defined as measures to reduce the prevalence of the disease.

Organisation of the Study

This thesis is of five chapters. An introduction in Chapter One describes the basis and significance of the study. It contains important sub-sections like the problem statement, aim, and objectives of the study.

In Chapter Two, the study reviews literature relevant to the study. It is mainly a compilation of academic publications discussing the prevalence and factors associated with malaria epidemiology, burden, and the life cycle of parasites, prevention, and control. The Chapter reviewed articles on genetic

diversity leading to polygenic infections and drug resistance focusing on the prevalence of mutations in *Pfcrt*, *Pfmdr1*, *Pf-exonuclease*, *Pfdhfr*, *Pfdhps*, and *Pfkelch13* genes.

Chapter Three, describes the materials and methods involved in this study. Sections under this chapter include research design, the study area, the population of the study, the sample size, the sampling technique, data collection method and instruments, molecular techniques applied, and data analysis techniques.

Chapter Four covered data analysis and contains graphical and tabular presentations of results. The chapter also contain the discussion of the results obtained.

Finally, Chapter Five consists of the novel findings, conclusion, and recommendation of the study and their implication in malaria control.

CHAPTER TWO

LITERATURE REVIEW

Background

Chapter two provides an overview of the life cycle of *P. falciparum* as well as a description of the global and local burden of malaria. Furthermore, the review looks at the genetic diversity of *P. falciparum* in the ecological setting, both previous and current antimalarial drugs, and SNPs in six known drug-resistance molecular markers.

Malaria burden

The genus *Plasmodium* is responsible for malaria (WHO, 2021). It is spread through a blood meal in sub-Saharan Africa by an infected female member of the *Anopheles gambiae* complex. Five species that infect humans are as follows: *P. falciparum, P. malariae, P. vivax, P. ovale* and *P. knowlesi* (Kori, Valecha, & Anvikar, 2018). Global data states that 99.7% of infections are caused by *P. falciparum* (Ofori et al., 2021). In endemic countries in the tropics, particularly in sub-Saharan Africa, where an estimated 93% of cases and 94% of deaths occur, malaria remains a cause of infection and death despite the development and implementation of the global action plan, intervention policies, and financial support for malaria eradication (WHO, 2020).

According to the World Health Organization's records, there were 219 million cases and 435,000 deaths in 2017, a 2 percent increase from the 90% reported in 2016. Additionally, 61% of malaria-related deaths occurred in children under the age of five (WHO, 2018). In the Sub-Saharan African region, malaria was found to be highly prevalent in Nigeria (27 percent), the

Democratic Republic of the Congo (12 percent), Uganda (5 percent), Mozambique (4 percent), and Niger (3 percent) (WHO, 2018). In 2019, the region was responsible for about 95% (~217 million) of the global burden of 228 million reported (WHO, 2020). In a similar vein, there was a rise in the number of infections in 2020, with an estimated 241 million cases and 627,000 deaths worldwide (Mahamar et al., 2022). The disease burden continues to compromise the quality of life in the tropics, particularly in pregnant women, their foetus as well as children less than five years of age and puts all of them at a greater risk of death (Mlugu et al., 2020; WHO, 2021). About 81.1 percent (77) of the study participants tested positive for *P. falciparum* malaria in Kenya (Touray, 2020) whilst the prevalence was 47.9% among outpatients in public hospitals in the Republic of Congo. Malaria was the cause of 18.4% of deaths and 64.8% of hospital admissions in Pointe-Noire, according to another study (Singana, Mayengue, Niama, & Ndounga, 2019). Recently, an estimated 10.8 million cases were recorded in Mozambique (Mayor et al., 2022).

In the past, malaria was responsible for 29% of deaths in the same country and 42% of deaths in children under the age of 5 years (Chidimatembue et al., 2021). A similar prevalence was reported in Burkina Faso with 1010 out of 1480 patients diagnosed malaria positive (Sondo et al., 2019). In other studies across Africa, 64.8% prevalence was recorded in Nigeria (Bajoga et al., 2019), 60.4 % in Gabon (Boukoumba et al., 2021), 98% in Burkina Faso (Yaro et al., 2021), and 19.2% in Tanzania (Mitchell et al., 2022). Furthermore, 32.54% was recorded in Gabon (Mba et al., 2022), 36.57% in the Central African Republic (Doutoum et al., 2019), 29 % of *P.falciparum* infection in Ethiopia (Negatu, Abebe, & Yalew, 2022) , and 55

% in Ibadan South West Local Government Area of Oyo State, Nigeria (Awosolu et al., 2021). It is currently estimated that about a third (11.6 million) of Africa's 33.2 million pregnant women were infected with malaria (Oboh et al., 2022) whilst 77% of all deaths worldwide, due to malaria are found in children under 5 years of age (Mahamar et al., 2022).

The burden of Malaria in Ghana

In Ghana, malaria is the leading cause of death and morbidity (NMCP, 2017; Tandoh et al., 2021). Studies have revealed that Ghana is a malaria-endemic nation with the disease occurring all year round in all sixteen (16) regions of the country. However, transmission varies by geographical region (Awine et al., 2017; PMI, 2020). In most of the northern part of Ghana, major transmission lasts within a six to seven-month period, particularly from July and November. In southern Ghana, malaria transmission lasts longer, peaking in May to June and from October to November (PMI, 2020). Due to their weak immune systems, children under the age of five and pregnant women in Ghana are at greatest risk for malaria (GSS, 2020).

According to the Ghana statistical service, malaria accounted for 28.1% of out-patient hospital attendance, 13.7% of hospital admissions, and 9.0% of maternal deaths among pregnant women in 2015 (Dun-Dery et al., 2021; GSS, 2017). On the other hand, about 34% to 38% of OPD cases and 27.3% of admissions were due to malaria (PMI, 2017). Currently, Dakorah et al., 2022 reports of the 41% of outpatients who are suspected of having malaria, 21% and 18% are confirmed among outpatients and inpatients, respectively (Dakorah et al., 2022). Additionally, out of the approximately 10.4 million cases of malaria recorded in 2016, pregnant women accounted for

1.4% of all malaria outpatient visits (NMCP, 2016). In a study elsewhere in Ghana, 59.2% (466/787) of 787 samples, tested positive for *P. falciparum* (Amoah et al., 2018) . In another study in the Greater Accra and Central regions of Ghana, parasite carriage ranged between 13.7% (13/95) and 64% (89/139) in Abura and 80% (64/80) and 96.4% (80/83) in Obom among asymptomatic persons (Adjah, Fiadzoe, Ayanful-Torgby, & Amoah, 2018). A prevalence of 73% was found in a study in the Ashanti region's Asante Akim North district (Heinemann et al., 2020) and 21.6% in Northern Ghana (Ahenkorah et al., 2020). In a recent study in 2021, involving three ecological zones, an overall prevalence of 16.1% by microscopy was recorded (188/1168) (Amoah et al., 2018). According to a study conducted in Ghana by Ejigu and Wencheko, prevalence of malaria by microscopy and rapid diagnostic tests was 20.63% and 27.82% respectively (Ejigu & Wencheko, 2021). Data across the regions also showed a prevalence range from 5% in Greater Accra to 30% and 31% in the Central and Eastern regions respectively (Ejigu & Wencheko, 2021). In 2016, Ghana contributed about 2% of the global malaria prevalence and 4% out of the 80% of global malaria deaths (WHO, 2017). Malaria has caused about 21.6 thousand deaths cumulatively from 2008 to 2019, particularly among children less than five years and persons between the ages of 15 to 49 years (Sasu, 2022). Almost all cases (98%) were of *P. falciparum* infections (Amoah et al., 2019; Dembele et al., 2021). Nonetheless, a prevalence of 87% *P. falciparum* and 13% *P. malariae* infected patients were recorded in the Volta region of Ghana (Sakzabre et al., 2020). Currently, with about 4% global prevalence (WHO, 2018) and 3% of global malaria morbidity, Ghana ranks among the 15 highest Malaria hotspot

countries (WHO, 2020). Figure 2 shows the distribution of malaria across Ghana.

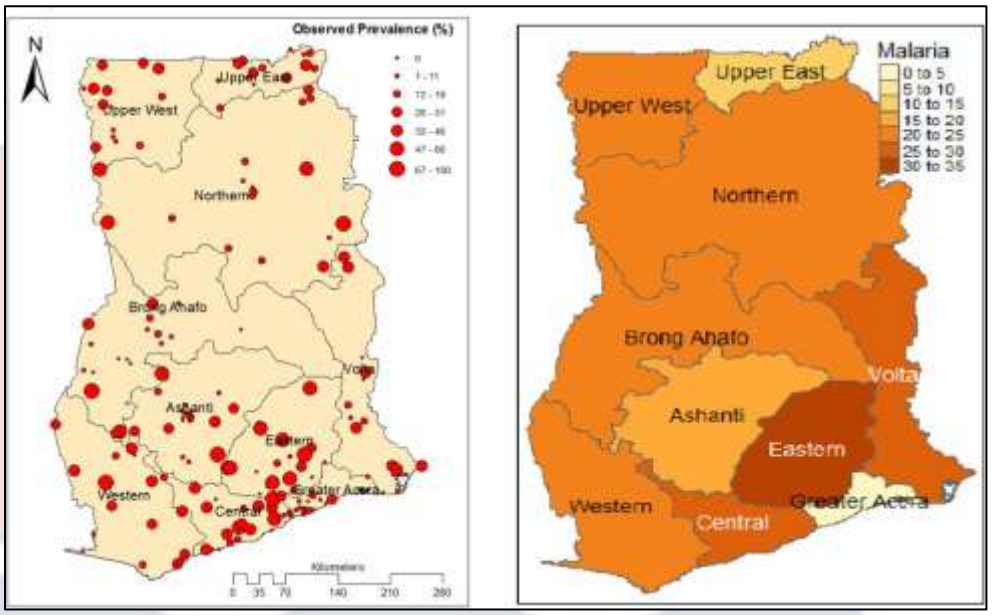


Figure 2: The distribution of malaria prevalence at survey locations (left panel) and regional estimates (right panel) in Ghana are depicted on a map of the country. Adopted from: (Ejigu & Wencheko, 2021)

In 2017, approximately 50.7% (950,776) of the population in the Central region reported with malaria out of 1,874,160 outpatient department attendants (OPD) (GSS, 2017). Recently, a community-based cross-sectional study in the Central Region's coastal zone found that the prevalence of malaria by microscopy and PCR was 10.6 percent and 27.1 percent, respectively, among elementary school students (Obboh, Okonu, & Amoah, 2020). Adjah and his colleagues also reported a malaria prevalence of 45.3% in Abura, a suburb of the Cape Coast Metropolis (Adjah et al., 2018). Currently, 45% of the estimated regional population (2,095,128) remains at risk of malaria (GSS, 2021).

Malaria control in Ghana has been progressive and the country is ready to target interventions based on epidemiological and entomological data at local levels irrespective of the variations within and between regions (i.e.,

district and sub-district) (PMI, 2020; WHO, 2021). However, current reports of more malaria cases in 2021, surpassing the figures of 2000, thwart the efforts of the last two decades towards the elimination of the disease and thus calls for more research to uncover current happenings.

Age distribution of malaria

Research across the WHO malaria regions asserts that Malaria prevalence is age-dependent (Mensah, et al., 2021) , with a high mortality rate associated with children under 5 years of age particularly in high transmission settings (Awosolu et al., 2021; Bajoga et al., 2019; WHO, 2020). Other schools of thought conclude that children aged more than 5 years have a higher risk of infection (Abate, Assefa, & Golassa, 2022; Collins et al., 2022) whilst clinical cases and parasitaemia are controlled in older individuals (Awosolu et al., 2021). The disparities here are mostly due to the level of immunity acquired against the parasites, and dependent on transmission settings (Rodriguez-Barraquer et al., 2018). In 2015, the prevalence of malaria in Markafi –Kaduna in Nigeria was 35.7% (419/1173) with the highest prevalence (19.3%) in the age group of 5 – 15 years (95% CI 17.0 - 21.6%), followed by the under 5 years group with 11.7% (95% CI 9.9 - 13.7%), whilst only 4.8% (95% CI 3.6 - 6.2%) of the total positive patients were adults. The study also observed that the difference in the proportion of positive cases in each age group was statistically significant (Chi-square $p<0.0001$) (Umaru & Uyaiabasi, 2015). In a study in Gabon, the most representative age group 15 to 49 years, recorded an incidence of 41.42% (3838 patients), followed by 0 to 4 years with 27.26% (2526 patients), age group 5 to 14 years (19.32%) and patients over 50 years with 12% (1111 patients) (Mba et al., 2022). Another

study in Mali also reported a higher incidence in children aged 6 months–5 years compared to those aged 6–10 years. Also, children aged 6-10 had a lower incidence in children older than 10 years (Coulibaly et al., 2021). The divergence in the age infection burden shows that malaria burden varies within geographic settings (Bello, 2021). Likewise, the age of persons has been implicated as a risk of malaria infection (Abuaku et al., 2021; Awosolu et al., 2021; Bello, 2021; Mensah., et al., 2021; Xia et al., 2020).

Ecology, climatic factors and malaria transmission in Ghana

Ghana is demarcated into three ecological zones with varying malaria transmission intensity. These are the Sahel savannah (Northern Ghana), the Forest zone (middle belt of Ghana), and the Coastal savannah (along the Gulf of Guinea) (Amoah et al., 2021). Figure 3 shows a map of Ghana indicating the malaria transmission zones and regions which fall within these zones. Currently, malaria surveillance is mostly of data from passive case detection (PCD) involving cases recorded at public facilities. Furthermore, results generalization from one study site for nearby communities is common and obscures the true prevalence and relationship between malaria and climatic factors (Ayanful-Torgby, Quashie, Boampong, Williamson, & Amoah, 2018). Also, seasonal malaria infections in the Northern regions, transmission all year round in the south, and varying prevalence across different ecological zones are evidence that malaria incidence is influenced by the ecological and climatic factors within different settings in the country (Awine, Malm, Peprah, & Silal, 2018; Forson et al., 2022). Infection with *P. falciparum* parasites were most common found in the forest zones of the Ashanti region (24.1%) and

least common in the coastal town of Ada (12.8%) in the Greater Accra region (Amoah et al., 2021).

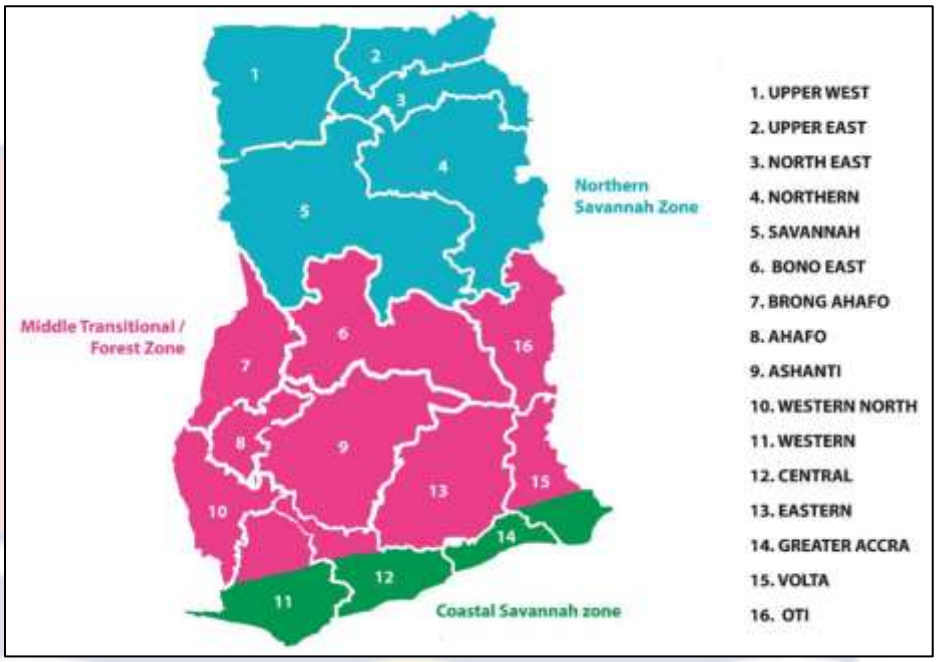


Figure 3: Ghana map showing the regions within each malaria epidemiological zone. Source:(Awine et al., 2018)

The Central region features more than two-thirds of forest ecology whilst the rest are coastal and transitional ecology (Kyere-Boateng & Marek, 2021). The prevalence of sub-microscopic asexual parasites was 59% out of 39 children who participated in a study in Cape Coast, Central region during the rainy season, whilst 70% of 37 children were recorded in Obom. However, the reverse was observed in the dry season with a higher prevalence of asexual parasite carriage in Cape Coast (72%) than in Obom (59%) (Ayanful-Torgby et al., 2018). In Ghana, very few studies have explored the dynamic impact of the ecology and its importance in malaria control. Such studies will provide an accurate profile of malaria transmission in different transmission settings.

The life cycle of the malaria parasites

The life cycle of the *P. falciparum* species occurs in two unrelated hosts - in man (intermediate host for asexual reproduction) and the female

Anopheles mosquitoes (definitive host for sexual reproduction). The infection is initiated by the bite of infected *Anopheles* female mosquitoes during a blood meal on a suitable host (humans). Motile sporozoites from the salivary glands of the mosquito are deposited into the skin and subsequently find their way into the blood capillaries of the host (Cockburn & Seder, 2018; Sato, 2021). Hepatocytes, or liver cells, are infected by the sporozoites, which migrate to the liver. This is known as the pre-erythrocytic or liver stage. Hepatic schizonts are the result of sporozoites in the liver multiplying asexually through schizogony (Sato, 2021). Hepatic schizonts rupture, releasing merozoites into the peripheral blood thus commencing the erythrocytic cycle (Sato, 2021). Typically, this period lasts between 8 and 15 days, depending on the *Plasmodium* species (WHO, 2018). *P. vivax* and *P. ovale* on the other hand, form hypnozoites (dormant/latent state) that reactivate to cause a relapse after several weeks to years of primary infection, sustaining transmission of these species (Ashley et al., 2018).

In the erythrocytic cycle, the merozoites infect non-infected circulating erythrocytes in the hepatic veins, reproduce asexually (intra-erythrocytic/asexual life cycle) and develop through the ring stage (trophozoites). Mature trophozoites divide asexually into intra-erythrocytic schizonts. When these schizonts mature, they release between 4 to 36 merozoites for each infected RBC over a 24-72 hour period in *P. falciparum* but differ in other *Plasmodium* species (Wahlgren, Goel, & Akhouri, 2017). A 48-hour asexual cycle has been observed in *P. vivax* and *P. ovale* spp., whilst *P. malariae* and *P. knowlesi* undergo a 72-hour and 24-hour cycle respectively. Some merozoites differentiate into male and female gametocytes

over 10–12 days. Gametocyte development is of five morphologically distinct stages. In the immature stage comprising stages I–IV, gametocytes are sequestered in the bone marrow and only mature stage V circulates in peripheral blood and remains in the peripheral circulation until ingested during a blood meal (Garrido-Cardenas, González-Cerón, Manzano-Agugliaro, & Mesa-Valle, 2019).

The sporogonic cycle describes the *Plasmodium* development in the mosquito. The mature male gametocyte (microgametocyte) divides into one to eight microgametes in the mosquito midgut, while the mature female gametocyte (macrogametocyte) grows into one macrogamete (Bennink, Kiesow, & Pradel, 2016). The macro and micro gametes fuse to form zygotes (diploid genome) in the midgut (Bennink et al., 2016). The zygote undergoes rapid meiotic division and mitosis, developing into a motile ookinete of numerous new haploid sporozoites within 18 to 36hrs later. The ookinete penetrates into the gut wall of the mosquito to become an oocyst (Siciliano et al., 2020). The oocyst matures, ruptures and releases sporozoites which migrate through the mosquito's midgut cellular wall and the hemolymph to the salivary glands and await introduction into a host during the next blood meal, thereby ensuring the continuous propagation of the *Plasmodium* parasite (Sato, 2021). Figure 4 shows the Life cycle of *P. falciparum* in humans and mosquitoes.

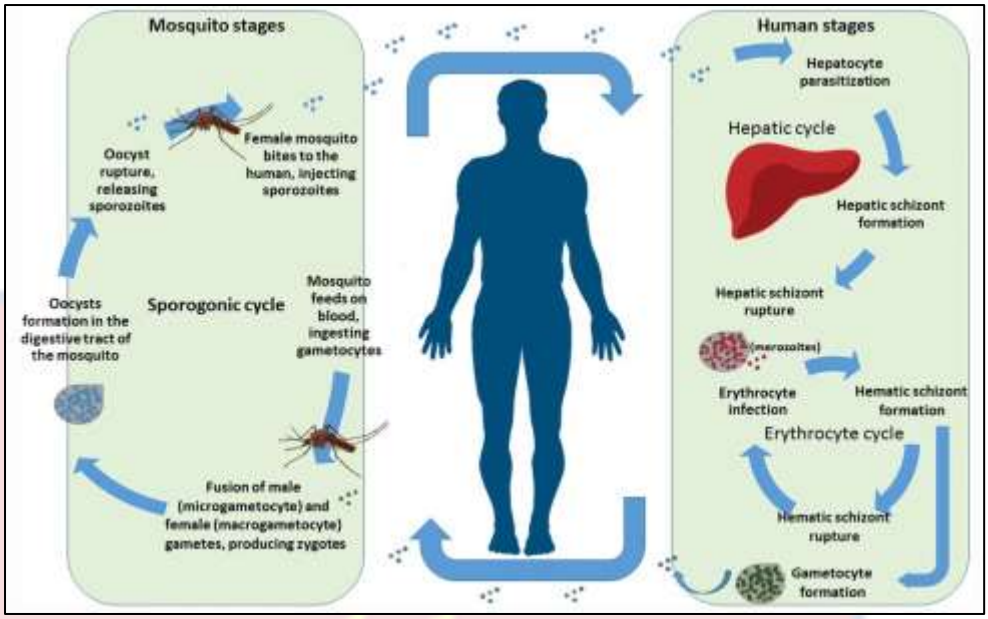


Figure 4: Sexual and Asexual life cycle of *P. falciparum* Source: (Garrido-Cardenas et al., 2019)

Signs and symptoms of malaria in human

Malaria infection can be classified as uncomplicated or complicated malaria. In general, the early presentation of malaria includes fever, chills, sweating, muscle or joint pain, and headache, which may occur every 48hours in the case of *P. falciparum, P. ovale,* and *P. vivax* infections, 24hrs in *P. knowlesi* and or 72hrs in *P. malariae* (CDC, 2019; Kori et al., 2018). These symptoms are a result of the continuous destruction of erythrocytes in the erythrocytic phase of the *Plasmodium* life cycle (Garrido-Cardenas et al., 2019). These presentations are common in uncomplicated malaria. The disease may progress to a severe state or complicated state, usually manifesting in organ failure, haematologic, and/or metabolic failure (CDC, 2019). Some recorded manifestations include, impaired weight gain in children, pulmonary oedema, renal failure, coma ('cerebral malaria'), splenomegaly, lactic acidosis, hypoglycaemia, shock and death (CDC, 2019). These presentations were thought to be limited to *P. falciparum* infections. However, some studies

have shown that *P. vivax* and *P. malariae* infections can cause such severe forms of malaria (WHO, 2016).

The distribution of *Plasmodium* parasites

The endemicity of human malaria parasites to a geographic location is influenced mainly by climatic conditions, invertebrate host, and human genetic factors such as the presence or absence of the Duffy factor, certain haemoglobin traits, and co-habitation of parasites during an infection, particularly in endemic areas (mixed infections) (Sato, 2021). *P. falciparum* is well distributed in both the tropics and subtropics and is responsible for the high incidences of malaria cases and deaths in these regions (WHO, 2017b). *P. vivax* is common in temperate zones with sporadic cases recorded in tropical areas. This is due to the fact that *P. vivax* thrives in cooler climates, at higher altitudes, and at lower temperatures (Baird, 2022; WHO, 2017b). Thus, it is prevalent in Central and South Americas (64%), the Mediterranean (40%), and south-eastern Asia (greater than 30%) but not in Ghana (Amoah et al., 2019; Brown et al., 2021). *P. malariae* is common to the African continent whilst *P. ovale, P. ovale wallikeri* (variant type) and *P. ovale curtisi* (classic type) are predominantly found in West Africa (Cohee & Laufer, 2017). The natural hosts, pig-tailed macaques, thrive in the forested regions of South East Asia and ensures the endemicity of where *P. knowlesi* in this area (Millar & Cox-Singh, 2015).

Gender distribution of malaria

Gender disparities in malaria distribution have been recorded in different settings. In Pakistan, 200 (88%) males and 26 (12%) females formed a total of 226 malaria-positive patients in a study (Hussain et al., 2021). A

study in Hubei Province, China reported that men (96.6%) constitute more *P. falciparum* malaria cases than females (3.4%) (Xia et al., 2020).

Across Africa such disparities have equally been recorded. In a study in Nigeria, males 19.4% (95% CI 17.1 - 21.7%) were more infected compared to females 16.4% (95% CI 14.3 - 18.6) and statistically significant (Fisher's exact = 0.0012) (Umaru & Uyaiabasi, 2015). However, males with a higher prevalence of 60.2% compared to their female counterparts 50.9% (Awosolu et al., 2021) as well as males (55%) and females (45%) (Ajogbasile et al., 2022) have been recorded in Nigeria. Okiring, in Uganda also reported a 70% prevalence among females (Okiring et al., 2022). Furthermore, Negatu and his colleagues in a study in Ethiopia observed that the risk of infection was higher in women (2.261 times) than in men (Negatu et al., 2022) whilst females (0.7 times) were less likely than males to develop a submicroscopic infection in Kenya (AOR: 0.74, 95% CI = 0.56–0.96, p = 0.025) (Otambo et al., 2022). In a very recent study in Gabon, females (58.91%) outnumbered male (41.09%) participants at a sex ratio of 1.56 (Mba et al., 2022). It is worth noting that, some studies in Zambia and Ghana, have demonstrated that pregnant women and women who have ever been pregnant are at a higher risk of *P. falciparum* infection due to the loss of their acquired semi-immunity than non-pregnant women (Chaponda et al., 2021; Völker et al., 2017).

In the Volta region of Ghana, the findings from a study by Sakzabre in 2020, show 69.07% infection in females and 30.93% in their male counterparts (Sakzabre et al., 2020). Another study in the Central and Eastern region of Ghana, recorded similar findings of high female prevalence (1986, 76.7%) compared to males (1669, 72.5%) (Mensah et al., 2021). Similarly,

the low prevalence in males (126 women and 77 men)is supported by a study in the Volta region of Ghana- state figures (Quaresima et al., 2021). In Ghana, Mensah and her peers reported an 18% higher risk of infection in men compared to women (Mensah, et al., 2021). However, Abuako reported that female patients were least prone to malaria parasites by microscopy (22.0%) or RDT (30.0%) compared with males (Abuaku et al., 2021).

Strategies for malaria control

The primary strategy for controlling malaria is a quick and accurate diagnosis, followed by effective chemotherapy treatment (Apinjoh, Ouattara, Titanji, Djimde, & Amambua-Ngwa, 2019; Ayanful-Torgby et al., 2018; Baghbanzadeh et al., 2020).

Malaria diagnosis

In areas where malaria is widespread, the most effective diagnostic tools are microscopy and the Rapid Diagnostic Test (RDT). Visualization of parasites in microscopes is the gold standard of diagnosis in many African countries (Berzosa et al., 2018). This method is sensitive, inexpensive, and useful in species identification and quantification. However, it has low sensitivity in detecting low parasite densities, limited in the differentiation of *Plasmodium* species and growth stages of the parasites (Amoah et al., 2019; Berzosa et al., 2018; Otambo et al., 2022). *Plasmodium* antigen-detection using RDTs is faster but is hindered by its low specificity and sensitivity, species differentiation, and inability to provide quantitation of parasite load (Amoah et al., 2019).

Molecular tools have become accessible and simple to use but are confined to research laboratories (Okell et al., 2018; Torres et al., 2018) due to

the complex infrastructure, equipment sophistication, reagents required, maintenance culture, and qualified personnel (Nsanzabana et al., 2018). The most sensitive method are the Polymerase Chain Reaction (PCR) detection and amplification of target nucleic acids, which can detect parasitaemia as low as 2–5 parasites/µl. PCR-based methods have been used in detecting antigenic polymorphic markers such as merozoite surface proteins (msp1 and msp2), glutamate-rich protein (glurp), apical membrane antigen (ama1), and Circumsporozoite protein (CSP). Nonetheless, it lacks sensitivity in detecting infections with parasites that are less common or are thought to be subject to strong immune selection (He et al., 2021; Nkhoma et al., 2018).

Malaria control in Ghana

Government efforts in malaria control began in the 1950s (NMCP, 2022). The mandate of the National Malaria Control Strategic Plan—NMCP, (2000-2010) was extended to 2020 to reduce malaria morbidity and mortality by 75 percent by 2020 (PMI, 2020). This resulted in an 85 percent reduction in malaria deaths from 2012 (2,275) to 2018 (417). That notwithstanding, malaria morbidity remains high nationally (PMI, 2020) with an unlikely ability to eliminate local transmission of the disease before 2030 (WHO, 2016).

Malaria control has involved healthcare facilities such as health centres, community health planning services (CHIPS), and community-based pharmacies, hospitals, laboratories, and research institutions in the country. These facilities are involved in the diagnosis, treatment, and surveillance of the disease. Some of their efforts are recorded in the implementation of malaria control and eradication interventions including; vector control

measures such as the use of long-lasting insecticidal net (LLIN), indoor residual spraying (IRS), and rapid diagnostic test (RDT) (NMCP, 2013), and seasonal malaria chemoprevention (SMC) with Sulfadoxine– pyrimethamine under Directly Observed Therapy (DOT) (Afutu et al., 2021; de Cola et al., 2022). The involvement of health facilities in diagnosis using RDTs augmented testing before treatment (WHO, 2018). It increased malaria testing from 39% in 2013 to 78% in 2016 (PMI, 2017). This resulted in a decline in malaria-related mortality from 19% in 2010 to 4.2% in 2016 (PMI, 2017). Additionally, the country adopted the Artemisinin-based Combination Therapy (ACT), in 2004 (MOH, 2009). The institution of the policy began with Artemisinin-amodiaquine but expanded since the last revision of the treatment policy in 2009. The ACTs in use in Ghana currently are, Artesunate Amodiaquine (AA), Artemether-Lumefantrine (AL), and Dihydroartemisinin Piperaquine (DHAP) (MOH, 2009). This treatment plan has worked for more than a decade and continues to be effective (Ofori et al., 2021).

Molecular genotyping

The *P. falciparum* parasite's genome has evolved from a single gene sequence into a complete genome sequence (He et al., 2021; Roh et al., 2019). To identify individual clones, epidemiological studies require a single, high-resolution molecular marker. However, epidemiological studies on drug resistance involve the genotyping and analysis of several SNPs in one or more genes coding for drug resistance to a drug of choice, mostly not indicative of clinical resistance (Koepfli & Mueller, 2017). Illumina and other highly sensitive and specific next-generation sequencing (NGS) technologies permit the examination of highly polymorphic molecular markers and have been

validated for the investigation of *P. falciparum*'s genetic diversity (He et al., 2021). Molecular epidemiology has reduced the time frame in identifying resistant markers (Ndwiga et al., 2021) particularly, in the biannual (every 24 months) surveillance as recommended by the WHO (WHO, 2019).

Selective whole genome amplicon (sWGA)

Selective whole genome amplicon sequence (sWGA) relies on the principles of NGS run on Illumina sequencing platforms. Such technologies are expensive but are the most reliable options due to their high sensitivity and specificity making it practical for malaria genomics studies using DNA collected from dried blood spots (DBS) rather than venous blood (Ibrahim et al., 2020; Neafsey et al., 2021; Oyola et al., 2016). In recent applications of sWGA, *P. falciparum* DNA yield was 18-fold more when compared to results using nonselective whole genome amplification (Oyola et al., 2016). Contamination of *Plasmodium* DNA with human genomic DNA has been the main challenge in sequencing approaches. However, sWGA uses selected short oligonucleotide probes of 8–12 primers that target species-specific DNA motifs present in the genome of interest but absent in the genomes of other species, limiting investigations to predefined loci of interest (Taylor et al., 2015). sWGA also employs a unique strand-displacing phi29 DNA polymerase enzyme which can displace complementary strands as it amplifies DNA from the primers (Aninagyei et al., 2020; Oyola et al., 2016). sWGA in Illumina genotyping permits sample multiplexing thus enabling the detection and quantification of parasite variants and minority clones (low-density clones) in multiclonal infections. Multiplexing involves the use of PCR primers and molecular inversion probes generated from well-characterized

microsatellite regions and SNVs (Single nucleotide variants) that vary geographically to generate "molecular barcodes" (Early et al., 2019; Hemming-Schroeder et al., 2021; Otambo et al., 2022; Oyola et al., 2016). Thus, multiplexing further allows the differentiation of samples from different geographic origins, ecological zones, and interventional settings. It can also be used to trace the historical spread of phenotypes of *P. falciparum* (Early et al., 2019; Hemming-Schroeder et al., 2021; Otambo et al., 2022).

Molecular Diagnosis in Ghana

In early 2010, WHO issued revised treatment guidelines that called for a shift from presumptive to test-based approach in malaria diagnosis (WHO, 2009) and it was adopted in Ghana (Orish et al., 2016). Over the years, there has been an increase in the use of both microscopy (the gold standard) and RDTs which has helped increase diagnosis and management of malaria but with limitations (PMI, 2017).

Molecular analysis is an advancement over the detection limit of the Light microscopy and rapid diagnostic tests (RDT) even though it is limited to the research institutes. In Ghana, the most widely used molecular method for the Characterization of local patterns of endemicity is the PCR-based protocols for parasite presence/absence determination. The use of PCR helped increase the diagnosis of malaria by 31.9% and 15% in asymptomatic P. falciparum infections in a seasonal field survey conducted in Ghana, that would otherwise have been lost due to limitations in the conventional diagnostic tools being slide-negative by microscopy (Tiedje et al., 2017).

Apart from the benefit PCR in diagnosis, the technique has also been employing in the molecular determination of drug resistance in the country. In

2007, nested mutation specific PCR was used for the detection of Pfcrt T76 and Pfmdr1 Y86 alleles highlighting the significance of mutations in the evolution of chloroquine resistance. The study formed the baseline for molecular markers profile for Ghana and was partly used to support the decision by the Ghana Malarial Control Program to replace chloroquine with a combination of amodiaquine and artesunate as the first-line drug (Duah et al., 2007).

Over the years, PCR assays have been used in estimating asymptomatic malaria prevalence in blood donors in the Volta, central, Ashanti and Greater Accra regions of Ghana (Muntaka & Opoku-Okrah, 2013; Owusu-Ofori, Gadzo, & Bates, 2016; Siakwa et al., 2014). In similar studies, Duah and her team in 2016 determined the genetic diversity of malaria parasites in Ghana by detecting the presence of msp2 alleles in P. falciparum isolates from nine sentinel sites in Ghana (Duah, Matrevi, Quashie, Abuaku, & Koram, 2016). Another study by Agyeman-Budu and colleagues investigated parasite diversity in asymptomatic infections in the forest belt area of Ghana (Kintampo) and found a predominance of 3D7 over FC27 at a ratio of 4:1 in the dry season (Agyeman-Budu et al., 2013). Recently, both Adjah and Abukari have determined the seasonal variations and the diversity and MOI of P. falciparum using polymerase chain reaction (PCR)-based genotyping of microsatellite markers (Abukari et al., 2019; Adjah et al., 2018).

Currently, genetic variants of P. falciparum are being typed, with the aim of describing parasite population dynamics and the identification of vaccine targets. This involves the determination of allele prevalence of variable Plasmodium genes in different human populations exposed to

malaria, including community-based comparisons and the determination of clonal multiplicity in individuals (Dakorah et al., 2022; Matrevi et al., 2022) . In 2015, a genome-wide sequences analysis of isolates from two ecologically distinct areas in Ghana showed the genetic structure of parasite populations as very similar (Duffy et al., 2015) while a similar studies have identified novel mutations in the Ghanaian parasite population (Aninagyei et al., 2020;Matrevi et al., 2022). In a recent study, Dakorah and her team found ecological and seasonal differentiations in plasmodium species in the central region using selective whole genome sequencing (Dakorah et al., 2022).

The observations made in these studies Could be consistent with either 1) soft selective sweeps at locus studied or 2) by recombination over the decade. However, ecological and seasonal influences may play an underlying role. Thus, Understanding the molecular basis of the persistence of the plasmodium falciparum is therefore important for malaria control efforts Thus, further studies in this area are warranted.

Genetic diversity in *P. falciparum*

The variations in the genetic makeup of *Plasmodium* species within a species population are referred to as genetic diversity. During the mosquito vector's sexual development, genetic recombination occurs and is said to be responsible for genetic diversity. Furthermore, within endemic zones, variations in parasite prevalence are caused by the diversity of malaria parasites in various individuals, populations, transmission settings, and seasons (Kar et al., 2016; Singana et al., 2019)

Complexity of infection

P. falciparum infections in endemic settings are usually characterised by the co-existence of multiple yet genetically distinct parasite clones in a susceptible human host (Abukari et al., 2019). Figure 5 shows a diagram of the complexity of infection.

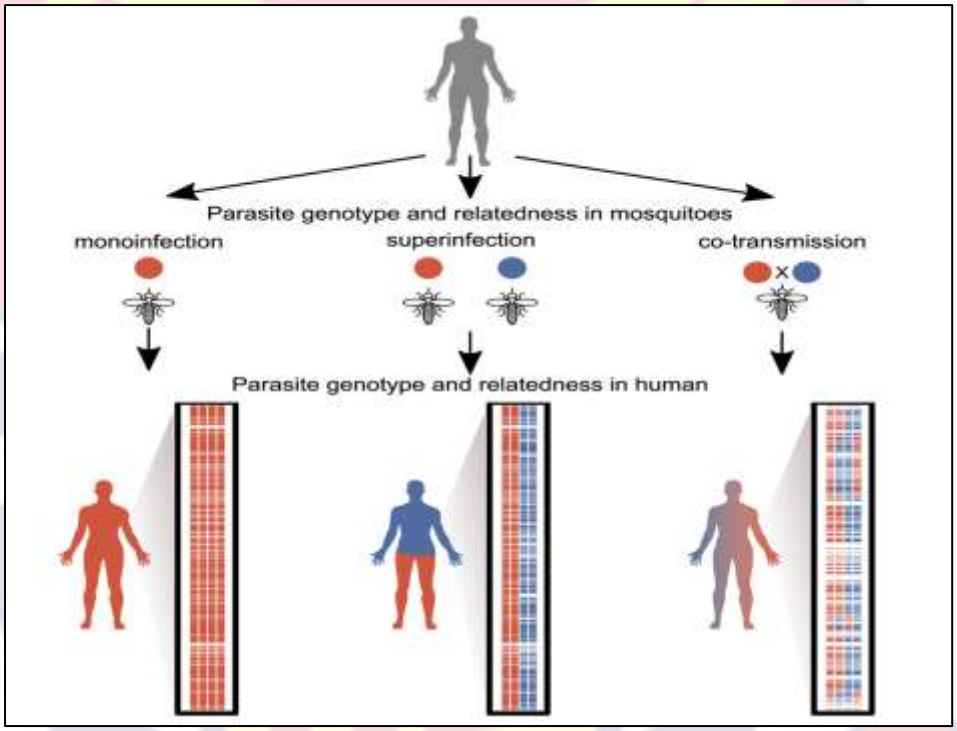


Figure 5: Diagram showing the complexity of infection
Source: (Nkhoma et al., 2020)

The transfer of genetically distinct sporozoites from a single bite during a blood meal is thought to be the cause of this phenomenon, which is referred to as the complexity of infection (COI) or multiplicity of infection (MOI) (Adjah et al., 2018). Monoclonal infections are those caused by parasite genes that share a common allele at each of the allelic family loci, whereas polyclonal infections are caused by genes that share multiple alleles (Touray, 2020). Immunity to multiple strains and the emergence of highly virulent and drug-resistant parasite strains, both result from infections with multiclonal infections (Sondo et al., 2019; Touray, 2020). Recombination between

genetically distinct clones, which alters the genetic diversity of the parasite population, is hypothesized to have a direct correlation with the intensity of malaria parasite transmission. This parallel relationship is sometimes referred to as a "genomic thermometer" (Roh et al., 2019), with numerous studies asserting that genetic recombination between parasites decreases as transmission reduces leading to a low COI/MOI and vice versa (Roh et al., 2019; Sarah-Matio et al., 2022)

The complexity of infection using Likelihood (COIL) has most commonly been used in the analysis of COI, relying on allele frequencies from monoclonal samples (Galinsky et al., 2015). Malaria genomic analysis, on the other hand, is complicated by the presence of multiple genetically distinct parasite lineages that may differ in terms of geographic origin, drug resistance profile, or other temporal variations or characteristics of interest. The use of Markov chain Monte Carlo, popularly known by its acronym THE REAL McCOIL is a Bayesian approach that estimates allele frequency and COI using Single Nucleotide Variants data from polyclonal parasite samples by turning heterozygous SNP data into robust estimates of allele frequency (Chang et al., 2017). McCOIL has proved to be a formidable method in addressing this challenge. Even though, the impact of MOI on malaria epidemiology is undefined (Sarah-Matio et al., 2022), it may be an important element in malaria control due to its influence on genetic diversity (Sondo et al., 2019). A study in China, found mixed-species infections in 15 out of the 93 malaria episodes (~16%) with the number of haplotypes ranging from 1 to 6 (He et al., 2021).

The complexity of infection in Africa

Malaria infections involving multiple strains have been reported across the African continent. A study in 2019, reports a high prevalence of polyclonal *P. falciparum* infections in Eswatini, after genotyping samples from both symptomatic and asymptomatic patients (Roh et al., 2019). Based on polymorphic regions of the msp1 and msp2 genetic loci, another study in Burkina Faso found that all children between the ages of one and seven in the rural area and between the ages of one and six in the urban area had at least 90% of multiple infections (Soulama et al., 2009). However, there was no significant difference ($p = 0.3$) in the frequency of multiple infections between urban 96.0 percent (92.0–100) and rural 92.0 percent (86.3–97.7) regions (Soulama et al., 2009). Similarly, a frequent occurrence of multiple infections is found in patients infected with *P. falciparum* in Nanoro (Sondo et al., 2019). A study conducted in western Kenya using ten microsatellite marker loci found a polyclonal infection prevalence of 79.69 percent, though the majority of alleles were found at low frequency (Touray, 2020). In Tanzania, 50% of the isolates were found to be polyclonal (COI > 1) (Morgan et al., 2020) whilst 64.5% of individual samples were polyclonal in Congo (Miller et al., 2017) and 1.45 in Sudan (Mustafa et al., 2017).

The complexity of infection in Ghana

As a country with a high prevalence of malaria (MOH., 2014), Ghana is likely to experience genetic recombination in the parasite landscape, similar to observations in other malaria-endemic areas (Amambua-Ngwa et al., 2019). The presence of multiple parasite clones may facilitate parasite evolution, and enhance the chance of survival, thus advancing the persistence of the disease

in the country. Despite this, very little is known about Ghana's genetic diversity of malaria parasites. At least 55% of *P. falciparum* infections in 15 African countries were polygenetic, with up to nine clones in some infections from Ghana, Guinea, and Malawi, according to a genome-wide study (Amambua-Ngwa et al., 2019). In Ghana, Duffy and his colleagues reported close similarities in the genetic makeup of parasite populations in two ecologically distinct areas (Duffy et al., 2015). In contrast, Duah in 2016, found a high genetic diversity within the msp2 gene, however, their report did not correlate with differences in parasite variants across the country (Duah et al., 2016). These disparities are likely a result of gene flow due to human movements and methods employed in the study. In another study using microsatellite analysis in Ghana, the diversity and MOI of *P. falciparum* parasites in non-symptomatic volunteers living in Obom (high malaria transmission intensity) was higher compared with that in Asutsuare (low malaria transmission intensity) (Abukari et al., 2019). In a recent study, 12 (10.1%) of infections were found to be monoclonal whilst 107 (89.9%) were biclonal or polyclonal after analysing 119 *P. falciparum* infections across three ecological zones with samples from the Forest zone (Konongo) having the highest number of different parasite clones compared with samples in the Coastal zone (Ada) (Amoah et al., 2021). Another study in Ghana recorded up to four (4) genotypes per infection (Matrevi et al., 2019) whilst another report of a (227) 55.5% prevalence of polyclonal infections in their study (Tandoh et al., 2021). Meanwhile, An average of 3.1 was recorded in other parts of Ghana (Lamptey et al., 2018).

Impact of ecological zones and climatic factors on Genetic diversity of *P. falciparum*

Studies attribute the persistent transmission of malaria in Sub-Saharan Africa (SSA) to changes in biological, ecological, and climatic factors that control the life cycles of both vector and parasite and sustain malaria infection all year round (Mohammed et al., 2022; Rotejanaprasert, Lee, Ekapirat, Sudathip, & Maude, 2021; Short, Caminade, & Thomas, 2017)

Genetic diversity of *P. falciparum* in ecological zones

Malaria prevalence varies based on the transmission intensity in a geographical setting. Malaria genomics can be used to determine how much malaria transmission is occurring in a given area by measuring transmission intensity using population-level COI estimates (Kar et al., 2016). High transmission areas are classified as holoendemic or hyperendemic (prevalence is usually >50%), with holoendemic areas having intense transmission all year-long, whilst hyperendemic observe intense seasonal transmission (Birhanu, Yihdego, & Yewhalaw, 2018). Moderate transmission areas are classified as mesoendemic (prevalence of 10%-50%) with regular seasonal transmission. Low transmission areas are classified as hypoendemic (prevalence <10%) with, usually seasonal or intermittent transmission (Birhanu et al., 2018). These groupings have resulted from multiple indicators used to identify the transmission intensity and/or burden of disease in localities (Birhanu et al., 2018).

Studies in the WHO regions have used different techniques including size-polymorphic antigens and microsatellite markers (Zhong et al., 2018). Others have used geographic information systems (GIS), the incidence of

malaria, the carriage of asymptomatic parasites, reported fever, serological responses to malaria-specific antigens, the abundance of mosquitoes, exposure to infected mosquitoes, and age-adjusted prevalence to describe malaria transmission and classify its endemicity (Allotey, Osae, Mohammed, Kotoh, & Kenu, 2021). Notwithstanding, the identification of such patterns of transmission could be a challenge due to the diverse ecological niches unique to every setting (Baghbanzadeh et al., 2020).

Infection with multiple parasite clones facilitates higher recombination rates within the mosquito, leading to shorter haplotype blocks and decreased linkage disequilibrium (LD - the non-random association of alleles of different loci) within parasite populations. Population-level COI estimates are the most responsive to short-term changes in transmission intensity, such as a successful malaria control program, whereas LD estimates are the most representative of a parasite population's transmission intensity over time. Malaria can be detected in settings with low COI and high transmission intensity using the correlation between COI and transmission intensity (Morgan et al., 2020). Some studies in various ecological settings demonstrate that endemicity varies across small eco-climatic scales and between ecotypes (Amoah, et al., 2021; Awine et al., 2018; Kar et al., 2016; WHO., 2016) as observed in Nigeria (Bello, 2021), Kenya, Madagascar (Howes et al., 2016; Rice et al., 2021) and India (Ranjha & Sharma, 2021). People living in or near forest zones in endemic areas, according to some findings, are more likely to get malaria (Kar, Kumar, Singh, Carlton, & Nanda, 2014). Other reports of high malaria incidence in forested areas demonstrate that the parasites thrive better in these zones due to favourable climatic conditions (Kar et al., 2016;

Otambo et al., 2022). Low prevalence of malaria among coastal district have been reported in India (Pradhan & Meherda, 2019).

Genetic diversity of *P. falciparum* and climatic factors

Changes in Climatic factors affect the dynamics of malaria as temperature, humidity, and rainfall, influence vector prevalence, development, biting rate, and parasite survival (DePina et al., 2019; Endo, Yamana, & Eltahir, 2017; Le, Kumar, Ruiz, Mbogo, & Muturi, 2019). For example, factors such as temperature (between 18–30 ◦C) and humidity (60–90%) favours the breeding of malaria vectors (Segun, Shohaimi, Nallapan, Lamidi-Sarumoh, & Salari, 2020). Rainfall, its saturation or absence can influence the survival and distribution of mosquitoes, causing a reduction in the number of mosquito vectors by sweeping away eggs and larvae in breeding sites or influencing the temperature and humidity required for the survival of the matured vector (Segun et al., 2020).

Studies in the southern part of Mali report that the prevalence of infection during the rainy and dry seasons, varied from 85% to 55%, respectively (Koita et al., 2012). Another study, in Bolifamba, Cameroon showed that the malaria parasite was prevalent all year round but high in the rainy season (51.1%, 284/567) compared with the dry season (44.2%, 392/887) (Nkwescheu et al., 2015). In Burkina Faso, *P. falciparum* prevalence was low in the wet season (13.7%) than in the dry season (17.8%) (Yaro et al., 2021). Two other studies in Nigeria attributed the higher prevalence of malaria infection to the high rainfall in their study sites (Bajoga et al., 2019; Benjamin, Inabo, Doko, & Busayo, 2021). Also, the rainy season and its association with high malaria prevalence have been reported in Western Ethiopia (Haileselassie

et al., 2022), in Yogyakarta (Lestari, Lusiyana, & Nurochmah, 2020). However, other studies have reported high prevalence in the dry season (Coulibaly et al., 2021) whilst studies in Kenya found variability based on the study sites (Otambo et al., 2022).

The tropical wet and dry climate in the Central Region of Ghana creates a favourable environment for malaria transmission in its forest and coastal ecological zones. However, there is little data on malaria endemicity in the diverse zones in this endemic area (Ayanful-Torgby et al., 2018). Thus, the use of molecular methods in estimating the parasite prevalence in the communities during the rainy and dry seasons in the Central Region can provide the accurate impact of the climatic factors that affect the infection prevalence and COI in this malaria endemic area. Also, climatic conditions vary significantly within national boundaries and may influence microclimatic factors at the local borders, limiting the impact of national policies that assume universality in malaria control (Mohammed et al., 2022; Rotejanaprasert et al., 2021).

Genetic variation associated with antimalarial resistance

Genetic changes involved in the development of drug resistance account for the inefficacy of antimalarial drugs. Resulting polymorphisms, such as single nucleotide polymorphisms (SNPs), a combination of SNPs, or gene copy number variation in drug target genes have been reported in different studies. An increasing prevalence of resistant allele markers can provide an early warning of developing resistance, and a decreasing prevalence may indicate the return of sensitivity after a drug has been withdrawn (Okell et al., 2018). Table 1 shows the major SNPs associated with antimalarial drug resistance.

Table 1: Major SNPs associated with antimalarial drug resistance

Antimalarial derivatives	Chemical family	Drug name	Mechanism of action	Genetic markers for drug resistance	codons association with therapeutic failure	Molecular markers	References
Quinoline derivatives	4-aminoquinolines	Chloroquine	Accumulate in the digestive vacuole of parasite. Inhibition of heme detoxification	Point mutations in *Pfcrt*	*Pfcrt* 72–76 (CV**IET** and **S**VMN**T)**	*Pfcrt* K76T	(Fidock et al., 2000) (Djimdé et al., 2001)
		Amodiaquine	Accumulate in the digestive vacuole of parasite. Inhibition of heme detoxification	Point mutations and copy number variation in *Pfmdr1*	*Pfmdr1*: codons 86Y, Y184 and 1246Y	*Pfmdr1* 86Y and *Pfcrt* 76T	(Duraisingh & Cowman, 2005)

Pfcrt- Plamodium falciparum Chloroquine resistance transporter *gene*, 72-76- Codons for amino acid positioning on *Pfcrt* gene, K76T- change of amino acid from Lysine to Tyrosine at position 76, CVIET and SVMNT- drug resistant haplotypes of *Pfcrt gene*

Pfmdr1- Plamodium falciparum multi drug resistance gene, 86, 184, 1246- Codons for amino acid positioning on *Pfmdr1*gene

Table 1: Major SNPs associated with antimalarial drug resistance …continued

Antimalarial derivatives	Chemical family	Drug name	Mechanism of action	Genetic markers for drug resistance	codons association with therapeutic failure	Molecular markers	References
		Lumefantrine	Accumulate in the digestive vacuole of parasite. Inhibition of heme detoxification	Point mutations and copy number variation in *Pfmdr1*	*Pfmdr1* N86,184F and D1246 or increased copy number	*Pfmdr1* N86 and *Pfcrt* K76	(Duraisingh & Cowman, 2005)
		Mefloquine		Increased copy number of *Pfmdr1*	Copy number of *Pfmdr1*	*Pfmdr1* N86,184F and D1246	(Duraisingh & Cowman, 2005)
		Piperaquine		*Pfmdr1*: codons 86Y, Y184 and 1246Y		*Pfmdr1* *N*86Y *Pfcrt* *K*76T	(Duraisingh & Cowman, 2005)

Pfcrt- Plamodium falciparum Chloroquine resistance transporter *gene*, K76T- change of amino acid from Lysine to Tyrosine at position 76, *Pfmdr1- Plamodium falciparum multi drug resistance gene*, 86, 184, 1246- Codons for amino acid positioning on *Pfmdr1*gene, *N*86Y – change of amino acid from Asparagine to Tyrosine at codon 86

Table 1: Major SNPs associated with antimalarial drug resistance …continued

Antimalarial derivatives	Chemical family	Drug name	Mechanism of action	Genetic markers for drug resistance	codons association with therapeutic failure	Molecular markers	References
Antifolate derivatives	Sulfa drugs	Sulfadoxine	Inhibits the dihydropteroate synthetase enzyme (*Pfdhps*)	Point mutations in *Pfdhps*	*Pfdhps* 437G 540E and A581G	K540E	(Roper et al., 2004) (Pearce et al., 2009)
		Pyrimethamine	Inhibits the dihydrofolate reductase (*Pfdhfr*)	Point mutations in *Pfdhfr*	*Pfdhfr* 51I, 59R and 108N	Triple mutant	(Basco, de Pécoulas, Wilson, Le Bras, & Mazabraud, 1995)
Artemisinin derivatives	Endopero -xides	Artesunate Artemisinin Artemether		Polymorphism in *PfKelch 13* Protein	*Pfkelch13 validated markers* F446I, N458Y, M476I, Y493H, R539T, I543T, P553L, R561H, P574L and C580Y	*Pfkelch13 C*580Y	(Ariey et al., 2014) (Aninagyei et al., 2020)

Pfdhps-Plasmodium falciparum dihydrofolate reductase gene, 437G 540E and A581G- codons and amino acid change of public health importance in *Pfdhps gene, Pfdhfr- Plasmodium falciparum* dihydrofolate reductase gene, 51I, 59R and 108N- codons and amino acid change of public health importance in *Pfdhfr gene, PfKelch 13- Plasmodium falciparum Kelch*13 gene.

Polymorphisms associated with CQ resistance

Polymorphisms in the *P. falciparum* Chloroquine resistance transporter (*Pfcrt*) gene are the most common cause of CQ resistance (Chidimatembue et al., 2021; Njiro et al., 2022), and act by increasing the efflux of CQ from the parasite's food vacuole reducing its concentration in the parasite digestive vacuole (Fidock et al., 2000; Roux et al., 2021). The most commonly reported *Pfcrt* mutations are observed in codons 72 to 76. Primarily mutation of *Pfcrt* K76T confers resistance to Chloroquine and is implicated in susceptibility to AL and ASAQ. About 15 haplotypes have been recorded as a result of mutations at codons 72-76 in the *Pfcrt* gene, but the wild-type haplotype CVMNK and the Chloroquine resistant haplotype CVIET and **S**VMN**T**, are frequent in Asia and Africa (Chidimatembue et al., 2021; Mairet-Khedim et al., 2021; Niba et al., 2021; Thomsen et al., 2013).

A resurgence of Chloroquine sensitive haplotypes has been reported in several studies since its withdrawal (Ocan et al., 2019). In a study in Cameroon, the researchers reported both CQS and CQ haplotypes as well as mixed haplotypes at a prevalence of 25.3% CVMNK, 47.3% CVIET, and 27.4% respectively among asymptomatic parasite carriers in 2013-14 (Apinjoh et al., 2017). However, in Zimbabwe, a high prevalence of *Pfcrt* K76T was reported in Chiredzi (64%), Kariba (82%), and Bindura (92%), respectively (Roux et al., 2021; Schleicher et al., 2018) Another study reported a prevalence of 97.3% (109/112) for *Pfcrt* 76K wild type, followed by 1.8% for 76T mutant (2/112), and 0.9% for K76T (1/112) (Mulenga et al., 2021). Other studies, reported undetectable levels of Chloroquine resistant haplotypes in east Africa (Wamae et al., 2019), Mozambique (Chidimatembue et al., 2021),

and North Uganda (Fukuda et al., 2021), since the mutant haplotype no longer serves as a selective advantage in the absence of CQ drug pressure. Similar results were found in studies in Cameroon where all 205 samples involved in a study were wild-type for *Pfcrt* and thus sensitive to CQ (Fontecha et al., 2021). In a study in North Western Nigeria 11(61.11%) isolates were CQR (CVIET), one isolate (5.55%) was CQR (CVMET) whilst six isolates (33.33%) was CQS (CVMNK) (Adam et al., 2021). A study in Uganda recorded a prevalence of Lys76Thr from 7% (44 of 675) between 2010–12 to 87% (364 of 417; p<0·0001) in 2016–19 (Tumwebaze et al., 2021). Similarly, studies in Zambia (Mulenga et al., 2021), Mozambique (Chidimatembue et al., 2021) and North Uganda (Fukuda et al., 2021) have recorded no resistant alleles among their isolates. A prevalence of 84.7% was recorded in Chad (Das, Datta, Beng, & Kiromat, 2022) , whilst 100%, 88.5%, and 62.29% in Cameroon (Fontecha et al., 2021; Tuedom et al., 2021) in DRC (Yobi et al., 2022) respectively.

Polymorphisms associated with CQ resistance in Ghana

In Ghana, 76T is typically selected for the Chloroquine resistant haplotype CVIET, which is of Asian origin (Aninagyei et al., 2020; Asare et al., 2021; Mensah et al., 2020) . Nonetheless, the re-emergence of CQS *P. falciparum* parasites has been observed in some regions in Ghana at varying prevalence (Asare et al., 2021) . Mensah and her colleagues observed a steady decline in the CQR mutant allele *Pfcrt* 76T and the CIET haplotype in Begoro and Cape Coast, respectively, in the Eastern and Central regions. They reported a prevalence of 95% wild-type *Pfcrt* K76 and haplotype CVMNK in Begoro, while 29% of the samples collected from Cape Coast still carried

Chloroquine resistance mutations in 2017 (Mensah et al., 2020). Also, another study in the Central region of Ghana found the prevalence of *Pfcrt* K76 at Cape Coast and Assin Foso, two of the largest districts in the region to be 71.74% and 65.22% respectively (Asare et al., 2021) . This was higher when compared with findings from his previous study in 2014 where the prevalence of *Pfcrt* T76 mutation was 80.4% in Cape Coast and 75.9% in Assin Foso (Asare et al., 2021; Asare et al., 2014)

Polymorphisms associated with Pyrimethamine

Six SNPs in the *P. falciparum* dihydrofolate reductase gene *(Pfdhfr)* have so far been found to confer global resistance to pyrimethamine (PRY). These are A16V, C50R, N51I, C59R, S108N, and I164L (Chaturvedi et al., 2021). The main mutation that confers resistance to pyrimethamine is the single mutation -S108N. The resistance level is raised when the mutations N51I and C59R are added. As a result, parasites with triple mutations (N51I+C59R+S108N) are more resistant than those with double mutations, whereas parasites with quadruple mutations have the highest level of resistance (Boukoumba et al., 2021). Several haplotypes resulting from these SNPs have been implicated in PRY resistance. These include (in bold): single (A16**V**, C50**R**, N51**I**, C59**R**, S108**N**, and I164L), double (C50**R**-N51**I**, N51**I**-C59**R**, N51**I**-S108**N**), triple (C50**R**-N51**I**-S108**N** (RIN); N51**I**-C59**R**-S108**N** (IRN), N51**I**-S108**N**-I164**L** (INL), C59**R**-S108**N**-I164**L** (RNL) and quadruple (N51**I**-C59**R**-S108**N**-I164**L** (IRNL) mutations (Chaturvedi et al., 2021; Svigel et al., 2021). The quadruple mutation, IRNL, has also been reported at very low frequencies (<1%) in some districts of Kenya (Lucchi et al., 2015), India (Lumb et al., 2009), Indonesia (Basuki et al., 2018) and Thailand (Kuesap,

Suphakhonchuwong, Kalawong, & Khumchum, 2022; Osoti et al., 2022) The double mutation (C59R-S108N) dominates the Middle East, Asia, and India. The triple mutation RNL is common in various regions of Malaysia (>85%) and India. However, the most prevailing *Pfdhfr* mutation in Asia (100%) and Africa (about 66%) is the triple mutation IRN haplotype (Chaturvedi et al., 2021; Kuesap et al., 2022). Such high numbers of molecular markers of resistance for pyrimethamine (PYR) have also been reported in Angola (Ebel, Reis, Petrov, & Beleza, 2021), Burkina Faso (Cisse et al., 2017), Democratic Republic of Congo (DRC) (Kayiba et al., 2021), Nigeria (Fagbemi et al., 2020), Uganda (Conrad & Rosenthal, 2019; Uwimana, 2020) and Zambia (Chaponda et al., 2021). Apinjoh et al., (2017) in 2013 and 2014, also observed a high prevalence of S108N (100%) whilst both N51I and C59R recorded 99% among the *Pfdhfr* mutant alleles. However, pyrimethamine resistance was observed in Somalia with a 70% prevalence of the double mutant N51I-S108N and a 30% prevalence of the triple mutant IRN (Warsame et al., 2017).

The *Pfdhfr* **IRN** triple mutation of approximately 97% has been recorded in Angola (Ebel et al., 2021), 25.7% in Burkina Faso (Cisse et al., 2017), in 763/772 (98.83%) and 674/724 (93.09%) of samples in Mfou and Tibati, respectively in the DRC (Tuedom et al., 2021), 68.8% Zambia (Chaponda et al., 2021) and 96.6% in haplotypes $V_{16}I_{51}R_{59}N_{108}I_{164}$(12.1%) and $A_{16}I_{51}R_{59}N_{108}I_{164}$ (84.5%) in Gabon (Boukoumba et al., 2021). Furthermore, 80.50% was observed among migrants from Africa to China (Yan et al., 2021) and 93.8% in Nigeria (Quan et al., 2020). Mutation at codon 164 (I164L) is implicated in resistance to Cycloguanil, the active form of proguanil (Quan et

al., 2020) mostly at low frequency <1% in some districts of Kenya (Lucchi et al., 2015), 87% in India (Lumb et al., 2009), 2% in Angola (Kaingona-Daniel et al., 2016), in 112 isolates from 2004 to 2006 and 2009 to 2012 in Indonesia (Basuki et al., 2018), 58% in Thailand (Kuesap et al., 2022) and up to 80% in Uganda (Asua et al., 2021).

In Ghana, the I164L mutation was reported at a frequency of 0.9%, 1/112 (Mama et al., 2022), and 12.3% among Ghanaian isolates found in migrant workers returning to Guangxi from Ghana (Zhao et al., 2020) . In contrast, this mutation was not recorded in Nigeria (Quan et al., 2020), Cameroon (Tuedom et al., 2021) nor Kpone and Mamobi in the Greater Accra region of Ghana (Tornyigah et al., 2020). The wild-type alleles are still recorded but of low prevalence (9, 6.5%) in Sudan (Hussien et al., 2020) and Ghana 6.5% (Tornyigah et al., 2020)

Polymorphisms associated with Sulfadoxine

Five point mutations (SNPs) in the *Pfdhps gene* are involved in resistance to Sulfadoxine: S436**A/F**, A437**G**, K540**E**, A581**G**, and A613**S/T** **(Svigel et al., 2021)**. Sequential addition of mutations in *Pfdhps* influences Sulfadoxine resistance (Yogavel et al., 2018). Several combinations of double (SGEAA, AGKAA, SGKGA), triple (AGEAA, SGEGA), and quadruple mutations (AGEGA) have been documented from isolates worldwide (Chaturvedi et al., 2021). The most prevalent *Pfdhps* haplotype with triple mutations was SGEGA (Chenet et al., 2017), whilst the most prevalent Single point mutations were SGKAA and SAKGA (Obaldia III et al., 2015). Triple mutations (34%) are most common in Asia and the Middle East, followed by double mutations (16%) and single mutations (12%) (Chaturvedi et al., 2021).

Mutations recorded in these regions include AGEAA, SGEGA, SGNGA, and SGEGA (Chaturvedi et al., 2021). A novel mutation (K540T) has also been reported in *Pfdhps* in Indonesia (Basuki et al., 2018). In India, single point mutations (S436A/F, A437G) are common in West Bengal at a prevalence of 30% (Chatterjee, Ganguly, Saha, Guha, & Maji, 2017). The double mutant FGKAA (S436F-A437G-K540-A581-A613) has also been reported at a prevalence of 40%, after the incorporation of ACT (Artesunate+SP) (Chatterjee et al., 2017). Triple mutations identified include AGEAA (S436A-A437G-K540E-A581-A613) (Sarmah et al., 2017), SGNGA (S436-A437G-K540N-A581G-A613) (Das, Chetry, Kalita, & Dutta, 2016) . Quadruple mutations (AGEGA-S436A-A437G-K540E-A581G-A613) have been reported with approximately 20% prevalence (Sarmah et al., 2017).

The majority of countries in Africa have *Pfdhps* single point mutations (46%), followed by double (26%) and triple (6%) mutations (Chaturvedi et al., 2021). The single mutation SGKAA has been observed to prevail in Gabon (Boukoumba et al., 2021), Cameroon (Tuedom et al., 2021), DRC (Kayiba et al., 2021), Equatorial Guinea (Berzosa et al., 2018), in Chad (Das et al., 2022) and Nigeria (Quan et al., 2020). Unique point mutation, reported in Burkina Faso (A437G) (Cisse et al., 2017) and Liberia (A613S) (Yan et al., 2021) confers partial resistance (Berzosa et al., 2018). Double point mutations (SGEAA) are prevalent in Zambia (Hussien et al., 2020), Uganda (Ikeda et al., 2018), Tanzania (Bwire, Ngasala, Mikomangwa, Kilonzi, & Kamuhabwa, 2020), Kenya (Hemming-Schroeder et al., 2021) and Sudan (Bakhiet et al., 2019). Currently, the triple mutation (SGEGA) has been reported in Sudan and Tanzania (Kateera et al., 2016). Furthermore, mutations at position I431V

have been reported in Cameroon (9%), Guinea Bissau (0.6%) (Chauvin et al., 2015), and in Nigeria (Oguike et al., 2016). Apinjoh et al.,(2017) also recorded the *Pfdhps* I431V mutation at 16.3% and identified the novel K142N mutation at a prevalence of 8.5% (Apinjoh et al., 2017). A study by Yan in 2021 also recorded mutations in *Pfdhps* at all codons studied (I431V, S436A, A437G, K540E, A581G, and A613S), with single mutant haplotype (SGKAA; 62.66%) as the predominant haplotype among 13 haplotypes (Yan et al., 2021). Furthermore, the *Pfdhps* I431V has been observed at a prevalence of 13% in Cameroon, where together with other mutations in the *Pfdhfr*/*Pfdhps* alleles, formed an octuplet mutation N51I-C59R-S108N-I431V-S436A-A437G-A581GA613S (Chaturvedi et al., 2021; Sarah-Matio et al., 2022; Svigel et al., 2021). Studies in the greater Accra region of Ghana reported the absence of the wild-type alleles of (Aninagyei et al., 2020; Tornyigah et al., 2020). Grais and his colleagues did not detect the *Pfdhfr* I164L and *Pfdhps* K540E mutations touted with clinical resistance to SP in their study site Gabi. They reported a high prevalence of mutation at codons S436**A/F/G (65%)** and A437**G** (83%), whilst A581G and A613**S** mutations were rare (0.1–0.25) (Grais et al., 2018).

Polymorphisms associated with Sulfadoxine–Pyrimethamine combination

Seasonal malaria chemotherapy previously termed Intermittent preventive treatment with Sulfadoxine–pyrimethamine (IPTp-SP) is one of the key preventive interventions for malaria in pregnancy and seasonal treatment for children within endemic areas (Afutu et al., 2021; Mama et al., 2022). SP treatment failure in children resulted in the withdrawal of the drug. Nevertheless, in malaria-endemic areas, SP is still recommended for

intermittent preventative treatment during pregnancy (Mama et al., 2022; Roux et al., 2021), and for seasonal malaria chemoprevention (SMC) in children, SP is used in combination with Amodiaquine (SP-AQ) (Svigel et al., 2021). However, resistant markers of *Pfdhfr* and *Pfdhps* genes should not negatively influence the use of the drug, if its parasite clearance rate is not below 90% (Niba et al., 2021; WHO, 2015).

Specific multiple mutation combinations in the *Pfdhfr* and *Pfdhps* genes are involved in varying degrees of resistance to this antifolate combination (Roux et al., 2021). The combination of *Pfdhfr* triple mutant N51I, C59R, and S108N with the *Pfdhps* single mutant A437G results in the quadruple mutant responsible for the partial resistance to SP (Svigel et al., 2021; Tuedom et al., 2021). These mutants are common in Central and West Africa (Roux et al., 2021; Svigel et al., 2021). A combination of triple mutant *Pfdhfr* N51I, C59R, and S108N with *Pfdhps* double mutant GE results in a quintuple mutant implicated in clinical treatment failure and full resistance to SP (Svigel et al., 2021), and are however, common to East Africa (Roux et al., 2021; Svigel et al., 2021). The quintuple mutation leads to a 28-fold increase in Sulfadoxine resistance (Chitnumsub et al., 2020). The prevalence of the IRN-GE mutant in Malawi was>95% (Ravenhall et al., 2016), whilst Kenya, Mozambique, Nigeria, Tanzania, Uganda, and Zambia recorded a prevalence of >50% of the studied isolates. Also, three mutations in both *Pfdhfr* (IRN) and *Pfdhps* (GEG) resulted in a super-resistant sextuple haplotype (IRN-GEG) (Chaturvedi et al., 2021; Tuedom et al., 2021). The IRN-GEG mutants have also been recorded in Malawi at a 3% prevalence (Ravenhall et al., 2016) and 15% in Rwanda (Kateera et al., 2016). The occurrence of additional mutations

in the *Pfdhps* gene at codons A581G and A613S/T or *Pfdhfr* I164L to the quintuple IRN-GE haplotype results in sextuple mutants (*Pfdhfr*, N51I-C59R-S108N- *Pfdhps* S436A-A437G-K540E (IRN-AGE) as well as (*Pfdhfr*, N51IS108N- *Pfdhps* -S436A-A437G-K540E-A581G (IN-AGEG). These mutants have caused a decline in SP's IPTp efficacy and reduced the protection of infants in some East African countries (Kenya, Sudan Somalia) (Chaturvedi et al., 2021; Svigel et al., 2021). Researchers in Fougamou reported high rates of mutation in both *Pfdhfr* (>96%) *and Pfdhps* (Boukoumba et al., 2021). Sequencing the *Pfdhfr* gene's codons 16, 51, 59, and 108 and the *Pfdhps* gene's codons 436, 437, 540, and 613 revealed a prevalence of triple mutations, with *Pfdhfr* VIRNI and AIRNI accounting for 12.1% and 84.5%, respectively, and mutant haplotypes of *Pfdhps* SGEA, SGKA, and AGEA accounting for 37.9%, 25.9%, and 12.1%, respectively. Additionally, the percentage of quadruple mutants, IRN-A and IRN-G, was 20 and 93.1 respectively, while the percentage of quintuple mutants, IRN-GE and IRN-AE, was 57.8 and 5 respectively. However, the frequencies of codon mutations I51, N108, and I164 were 100% (116/116), whereas A16 was 91.4% (106/116), V16 was 8.6% (10/116) and R59 was 96.6% (112/116) (Boukoumba et al., 2021).

According to Das et al., (2022), the presence of mutations in the *P. falciparum* dihydropteroate synthase gene (*Pfdhps*) was found in nearly all samples (Das et al., 2022) . Also, in Das study, a low prevalence of 1.5% (5/348,) of samples carried wild-type alleles while, a large majority (285/336, 84.8%) of the parasites were of the 'triple' mutants with the 51**I**-59**R**-108**N** haplotype. Furthermore, a single mutation at position 436 (S436**A/C**) was

recorded at 53.4% (186/348, 95%CI 48.0–58.8%) of the parasites, when the *Pfdhps* gene was analysed. However, they recorded a low prevalence (8/336 overall, 2.4% [95%CI 1.2–4.6%] of the 'Triple' mutations of *Pfdhps* 437**G**-540**E**-581**G** (Das et al., 2022) .

When Yan and his colleagues analysed *P. falciparum* isolates from Chinese migrants from Africa, they found the N51I, C59R, and S108N mutant alleles at frequencies of 97.60, 87.43, and 97.01% in *Pfdhfr* among 5 haplotypes whereas a prevalence of 27.67% and 27.04% were recorded at the *Pfdhps* K540E and S436A (27.04%) respectively (Yan et al., 2021).

In two malaria hyper-endemic states in Benin (Klouékanmey and Djougou), the *Pfdhfr* triple mutant N51I, C59R, and S108N was the most prevalent haplotype (84.6%) and commonly associated with the *Pfdhps* A437G (50.5%) or the *Pfdhps* S436A and A437G (33.7%). The research also reported a low prevalence (0.8%) of the quintuple mutant, *Pfdhfr* IRN/*Pfdhps* GE (A437G and K540E) as well as mutations at codon A581G (2.6%), A613S(3.9%) and at codon I431V (3.9%) (Svigel et al., 2021).

Mutations at codon S436A, A437G, A581G, and A613S have been recorded by Mandoko in a septuple mutation (e.g., IRNL-GEG) and an octuple mutant (e.g., IRNI-VAGKGS) in the Congo (Nkoli Mandoko et al., 2018). In China, Twelve (12) unique haplotypes were found among 130 isolates with 65.38% (n = 85) carrying the quadruple allele combinations (CIRNI-SGKAA) responsible for partial resistance to SP (Yan et al., 2021).

Polymorphisms associated with SP resistance

An article from Ghana reported a 28% failure rate when SP was used for the treatment of malaria in children (Afutu et al., 2021). Whilst another

reported that mutation of Serine to Asparagine conferred about a 10-fold increase risk of treatment failure (Mama et al., 2022). In this study, only one isolate carried a mutation at codon 164 (I164L, 0.9%, 1/112), with no mutation detected at codon 540 (Mama et al., 2022). Also isolates carrying the triple *Pfdhfr* **IRN**I and double *Pfdhps* **A/FG**KAA were seen at a rate of 46.7% (49/105) whilst isolates of the quadruple mutation (**IRN**I-S**G**KAA) were 16.2% (17/105). The occurrence of other haplotypes, even though present were found at low frequencies between 1 and 8% (Mama et al., 2022). Isolates from children aged 2 to 14 who were diagnosed with malaria at municipal hospitals in Kintampo North Municipality (2012–2013 and 2016–2017), Accra (2016–2017), and Navrongo (2012–2013) were found to have a total prevalence of 88% of the Pfdhfr 51I, 59R (82%), and 108N (88%) mutant alleles (Abugri et al., 2018).

Polymorphisms associated with Artemisinin (ACT) resistance

The emergence of diverse *Pfkelch13* gene polymorphisms threatens malaria control and eradication efforts, as the ACT and its derivatives are the only available effective malaria treatment (Amaratunga et al., 2016; Ouji, Augereau, Paloque, & Benoit-Vical, 2018; WHO, 2018). Also, poor responses to treatment in some parts of Africa and South America have raised the possibility that these continents may be experiencing artemisinin resistance due to *de novo* emergence or the imported resistant genes (Maniga, Akinola, Odoki, Odda, & Adebayo, 2021; Nzoumbou-Boko et al., 2020; Zhao et al., 2021; Zupko et al., 2022) . Irrespective of these findings, some isolates without *Pfkelch13* mutations, have been implicated in ACT resistance in Cambodia (Mukherjee et al., 2017).

In vitro selections paired with current clinical epidemiological data concur to ACT resistance being established primarily by mutations in the beta-propeller domain of the *Pfkelch 13* protein (Siddiqui et al., 2020). Recent evidence suggests that these mutations result in reduced endocytosis of host-derived hemoglobin, and thereby decreased release of the ACT-activating moiety Fe2+-heme, thus reducing ACT potency (Birnbaum et al., 2020; Yang et al., 2019). It has been reported that each single nucleotide polymorphism in *Pfkelch13* enhances parasite survival during ACT chemotherapy, thus mediating different levels of ACT resistance (Ajogbasile et al., 2022). The presence of these mutations at allele frequency > 10% in a given geographical site indicates suspected Artemisinin resistance, and WHO recommends further investigation to confirm resistance (Dhorda, Amaratunga, & Dondorp, 2021; Ye, Zhang, & Zhang, 2022). In the initial observation of *Pfkelch13* resistance to ACT, C580Y, R539T, I543T, and Y493H mutations were strongly validated as conferring resistance to ART in the Greater Mekong subregion (Ippolito et al., 2021; Ndwiga et al., 2021), Cambodia (Kheang et al., 2017) and Vietnam (Thuy-Nhien et al., 2017).

Outside South East Asia, Papua New Guinea (Miotto et al., 2020) and Guyana (Mathieu et al., 2020) reported the independent emergence of *Pfkelch13*-C580Y mutation at low frequencies. Currently, the *Pfkelch13* validated markers include F446I, N458Y, M476I, Y493H, R539T, I543T, P553L, R561H, P574L and C580Y (Imwong et al., 2020) . Other markers have been categorised as candidate markers for Artemisinin resistance which include: P441L, G449A, C469F/Y, A481V, R515K, P527H, N537I/D, G538V, V568G, R622I AND A675V (Matrevi et al., 2022; WHO, 2020) . In

addition, a non-synonymous mutation, E252**Q**, has also been transiently associated with delayed parasite clearance along Myanmars borders in Thailand (Das et al., 2022).

Many studies around the world have reported *Pfkelch13* mutations with substantial geographic variation and prevalence (Ocan et al., 2019; Ye et al., 2022). Recently, R561H gene mutation has been recorded in Tanzania and Uganda (Asua et al., 2021; Bwire et al., 2020; Moser et al., 2021). However, there was no phenotypic expression to back its effect as a resistant marker. In another study, using CRISPR-Cas genome editing, the R561H gene mutation was implicated in ACT resistance in Rwanda, (Bergmann et al., 2021; Uwimana, 2020) and the C580Y in Uganda (Ndwiga et al., 2021). This highlights the local emergence of ACT resistance.

Four separate studies: Rwanda (Uwimana, 2020), Uganda (Asua et al., 2021; Conrad & Rosenthal, 2019), and Nigeria (Pacheco et al., 2019) have each identified the A675V mutation, whilst a study in Angola and Equatorial Guinea reported R539T and P574L among parasites (Yang et al., 2019). Another study in North Uganda also found polymorphism at codon 533 in the *Pfkelch13* gene previously reported in Cambodia to be responsible for ACT resistance (Maniga, Akinola, Odoki, Odda, & Adebayo, 2021). In Nigeria, five new mutations (F451L, N664I, V487E, V692G, and Q661H) out of the 13 SNPs were detected in the *Pfkelch13* gene with three of the SNPs (V692G, N664I, and Q661H), consistent with late parasitological failure (LPF) in the Enugu and Plateau States (Ajogbasile et al., 2022). Out of the 3299 beta-propeller domains sequenced from 11 malaria-endemic African countries, 36 unique non-synonymous mutations were found, including the M476I and

R561H mutations (Stokes et al., 2021). In Mozambique, a study by Gupta and his colleagues showed a low prevalence (< 1%) of four polymorphisms (L619L, F656I, V666V, and G690G) in the *Pfkelch13* gene (Gupta et al., 2018). Another study found V494I *Pfkelch13* polymorphism in Mozambique (Escobar et al., 2015). In a study in Chad, *Pfkelch13* SNP at codon 189 (K189**T**) was the most common whereas 4(1.1%) out of 349 samples had non-synonymous mutations at codons A578S, Q633R, V636A, and W660C in the propeller region (Das et al., 2022). Studies from neighbouring Togo (Dorkenoo et al., 2016) and Ivory Coast (Djaman et al., 2017) reported S522M A578S C532S and S522C respectively.

Interestingly no molecular markers have been implicated to confer resistance to artemisinin in Burkina Faso, even though a reduced efficacy has been observed in children aged 6–59 months (Gansané et al., 2021; Zupko et al., 2022) and in Nigeria, even though delayed responses to ACTs have been observed (Ajogbasile et al., 2022). Another study recorded 150 distinct alleles in the *Pfkelch13* gene in Sub –Saharan Africa with no evidence of any of the SEA ACT-resistant alleles. However, the study found SNPs currently designated as candidate markers (Ménard et al., 2016). Other studies in Southeast Asia (Maniga et al., 2021), Pakistan (Yaqoob et al., 2018), Burkina Faso (Zupko et al., 2022), and Nigeria (Ajogbasile et al., 2022) did not record validated markers among *P. falciparum* parasites.

Additionally, evidence from research has reported on the synergistic mutation in selected genes that allows the emergence of *K13* mutations (Ndong Ngomo et al., 2023). This is referred to as the parasite genetic background (PGB). The parasite genetic background consists of mutations

found in genes, such as P. falciparum ferrodoxine (Pffd- D193Y), P. falciparum apicoplast ribosomal precursor S10 (Pfarps-V127M and D128Y/H) protein, P. falciparum multidrug resistance protein 2 (Pfmdr2-T484I) and P. falciparum chloroquine resistance transporter (Pfcrt- I356T and Pfcrt- N326S). These mutations are expressed as a concatenated haplotype form (VDDNIT) as a reference allele (wild type) (Bwire et al., 2020). Mutations to the VDDNIT wild type haplotype may be linked to a change in the fitness of the P. falciparum strains and contribute to the development of the parasite's resistance to ART and a higher risk of developing severe malaria (Otienoburu et al., 2019)

Artemisinin resistance-associated polymorphisms in Ghana

In Ghana few studies have reported ACT resistance markers, these include M476I, P553L, and C580Y in two independent studies (Aninagyei et al., 2020; Matrevi et al., 2019; Mensah et al., 2020). Another study found C580Y and R539T among *P. falciparum* parasites among immigrants to China from Ghana (Huang et al., 2016). Variants of amino acid changes of codons associated with artemisinin (ART) resistance validated markers: I543I, I543S, I543V, R561P, R561R, R539I, and C580V have also been reported at low frequencies (<5%) (Matrevi et al., 2019). The most prevalent mutations, the K189T and A578S mutation have been reported in several African countries including Ghana but have equally not been associated with ART resistance (Apinjoh et al., 2017; Ndwiga et al., 2021; Ocan et al., 2019; Rodrigues et al., 2022). In a very recent scientific report, 78 unique mutations were observed only in Ghana, Including R404G, P413H, N458D/H/I, C473W/S, R529I, M579T/Y, C580R/V, D584L, N585H/I, Q661G/L (Matrevi et al., 2022). Over

200 non-synonymous mutations in the *Pfkelch13* gene have currently been identified worldwide (Maniga et al., 2021). However, a majority reported that African *Pfkelch13* gene SNPs have not yet been characterized even though new mutations are being reported (Matrevi et al., 2022). Thus, *Pfkelch13* mutants should be keenly monitored in Africa, where the disease burden persists, ensuring the continued efficacy of ACTs (Kayiba et al., 2021).

Polymorphisms associated with the *Pfmdr1* gene

A number of antimalarial medications, including Chloroquine (CQ), Lumefantrine (LMF), Amodiaquine (AQ), Mefloquine (MFQ), Quinine (QN), and Artemisinin (ACT), have been linked to the *P. falciparum* multi-drug resistance 1 (*Pfmdr1*) gene, which is also known as *P-glycoprotein homologue 1* (*Pgh1*). N86Y, Y184F, S1034C, N1046D, and D1246Y are the five most common *Pfmdr1* SNPs (Wicht, Mok, & Fidock, 2020). SNPs in *Pfcrt* (K76T) and *Pfmdr1* (N86Y) have also been linked in reports to strongly confer higher Chloroquine and Artemisinin resistance (Chidimatembue et al., 2021). In Africa, drug resistance is most likely caused by mutations N86Y, Y184F, and D1246Y, but not by S1034C or N1042D (Chidimatembue et al., 2021). However, sensitivity or resistance to the partner drugs Lumefantrine (LF) and Amodiaquine (AQ), select for different molecular markers in the same gene (Ajogbasile et al., 2022; Njiro et al., 2022). Several studies report that parasites with mutations at codon, N86Y and D1246Y select for resistance to CQ and AQ, but increased sensitivity to LF, MF, and ACT.

Reduced susceptibility to Lumefantrine is conferred by the *Pfmdr1* haplotypes N86, 184F, D1246, and *Pfcrt* K76 (Blasco, Leroy, & Fidock, 2017; Ehrlich, Bei, Weinberger, Warren, & Parikh, 2021; Huang et al., 2016; Ross &

Fidock, 2019). Also, there are reports that parasites harbouring the NFD haplotypes were able to tolerate higher (15-fold higher) Lumefantrine blood concentrations than parasites with the 86Y-184Y-1246Y haplotype (Ontoua et al., 2021). The NFD haplotype involved in Artemether Lumefantrine resistance has been observed in the Democratic Republic of Congo (Yobi et al., 2021), whereas all *Pfmdr1* mutant haplotypes were N86/184F/D1246 in a Cambodian study (Mairet-Khedim et al., 2021). A high prevalence of almost 50% (172/331) of the study isolates in Chad was found to have the NFD haplotype (Das et al., 2022), and 36.8% in Angola (Ebel et al., 2021). In a similar vein, a Wuhan study found that Y184**F** had a high prevalence of 47.17% and a low (4.72%) prevalence of N86**Y**, respectively (Cheng et al., 2021).

Several mixed infections have also been observed with the N(Y/F)D polyclonal haplotype recorded at a prevalence of 15(9.4%) (Zhao et al., 2021), Similar results were reported in two different localities in Mfou (1.45%) and Tibati (5.97%) in Cameroon (Tuedom et al., 2021). In Gabon, the 1246Y mutant allele was found to be prevalent, ranging from 1.3% to 6.7% among the districts and within different haplotypes (Maghendji-Nzondo et al., 2016), and 7.7% in Uganda (Achol, Ochaya, Malinga, Edema, & Echodu, 2019). The prevalence of the *Pfmdr1* 1246Y allele was 5.26% in Nigeria's Yobe state. However, this mutant was not recorded in Kaduna and Adamawa states (Adamu et al., 2020). Recently, a study in Mali hypothesizes a possible correlation between the gene and ACT susceptibility since N86Y point mutations declined after the introduction of ACT (Maiga et al., 2021; Tuedom et al., 2021). As a result, monitoring changes in the *Pfmdr1* SNPs may provide

early warning of the onset of ACT resistance (Al-Mekhlafi et al., 2022). Four *Pfmdr1* haplotypes were found in Cameroon, with 33.1% of the isolates carrying the wild-type alleles *Pfmdr1* N86, Y184 D1246. Their work also reported high mutation rates at codon *Pfmdr1* N86Y in both Yaounde (76%) and Mfou (84%) whilst the proportion of mixed genotype isolates was 14% in Yaounde and 12% in Mfou (Apinjoh et al., 2017). Grais found that resistance to amodiaquine was associated with a low prevalence of *Pfmdr1*, N86Y, 184Y, 1042D, and 1246Y in Niger (Grais et al., 2018). Chidimatembue et al. (2021) conducted a Mozambican study reported that the N86, S1034, N1042, and D1246 alleles were present in all pre-treatment samples. However, post-treatment polymorphisms were observed at codon 184 in this study: 184**F** in 43 (39.4%), Y184 in 42 (38.5%), and mixed Y/**F** in 24 (22.0%). The study also reported a low prevalence of the wild-type haplotype (NYD) compared to the mutant haplotype, (NFD) in their study sites (Chidimatembue et al., 2021).

A molecular study found that the N86 Y184F D1346-K76 haplotype was present in approximately half of all Chadian isolates (172/331, 52.0% [95% CI 46.4–57.5%] overall), while the **YY**Y**-T** haplotype was not found in any of the isolates. The N86**Y** mutation was also found in a small number of people (20/336, 6.0% [95% CI 3.7–9.0%]) (Das et al., 2022). Ruqayya Adam and her colleagues found that three sequences in the north-western region of Nigeria contained the mutation 184F, and that a fourth sequence was confirmed to be a double mutant (86Y-184F) (Adam et al., 2021). Six haplotypes were identified from 448 *P. falciparum* isolates taken from infected children in a comparable study: NYY, NFY, NFD, NYD, YFD, and

YYD. NFD was the most common haplotype among these (Ontoua et al., 2021),

Polymorphisms associated with partner drugs in Ghana (re look at it- contains non-Ghana articles)

In Ghana, Artemether-Lumefantrine and Artemether-Amodiaquine have been the primary treatments for mild malaria (Kamau et al., 2015). Nonetheless, mutations such as YFN (N86Y/Y184F/D1246N) have been observed in Greater Accra (Aninagyei et al., 2020) . In the central region of Ghana Asare et al., (2021) reported an overall prevalence of CQ-sensitive *P. falciparum* marker *Pfmdr*1 N86 to be 84.11%, with slight variations among his study sites – with a prevalence of 64% and 88.39% at Cape Coast (64%) and Assin Foso (88.39%) respectively (Asare et al., 2021).

Markers associated with Piperaquine resistance (Exonuclease gene)

In addition to commonly available ACTs such as artesunate-amodiaquine and Artemether Lumefantrine, Dihydroartemisinin–Piperaquine poses different selective pressure in sustaining parasite drug resistance (Diakité et al., 2019). By removing a single nucleotide monophosphate (dNMP) from the end of one strand of DNA and acting as a proofreader during DNA replication, the exonucleases contribute to the stability of the genome (Diakité et al., 2019). Recrudescence isolates from DHA–PPQ treatment failures in Cambodia contain a non-synonymous SNP, E415G, on an exonuclease-encoding gene (Amato et al., 2017). However other researchers disagree (Boonyalai et al., 2022). Mutations in *P. falciparum* exonuclease gene have been implicated as the culprit behind DHA–PPQ resistance in Mali (Diakité et al., 2019). *P. falciparum isolates* from Vietnam's Central Highlands

both contain the *Pfexonuclease*-E415G mutation, with prevalence rates of 66.7% and 85.5% in Dak Nong and Dak Lak, respectively (van der Pluijm et al., 2019).

Molecular studies on antimalarial resistance in Ghana

Antimalarial drug resistance thwarts malaria control efforts in Ghana. The national treatment guidelines have been adjusted severally to accommodate challenges encountered as parasites become resistant to treatments. Since 2009, artemisinin-based combinations have been the formal drug choice of treatment in the country. However, current reports indicate a low prevalence of resistant markers in the population (Aninagyei et al., 2020). Thus, national treatment and prevention policies can benefit from validated molecular markers associated with ACT and/or partner drug resistance (Ndwiga et al., 2021; WHO, 2018).

Conclusion

The burden of malaria on the health of Ghana's population, the suitable weather conditions for the survival of both vector and parasite, and the diverse intertwined ecological niches of the country, create a challenge in malaria eradication, particularly in the country's uniform implementation of malaria control measures. Since malaria elimination includes the interruption of local transmission (WHO, 2017b), community-based prevalence studies complemented with molecular surveillance across the length and breadth of the country cannot be overemphasized. These epidemiologic tools will aid in assessing malaria control measures and programmes, determining current malaria burden and parasite genetic changes, which can negatively influence control efforts, as well as provide the required knowledge for a better

understanding and implementation of targeted intervention for malaria control and eradication in individual settings. The quantification of the genetic diversity, in an infection in distinct geographic locations across changing malaria seasons, provides evidence of the nature and extent of genetic diversity within the circulating species (Bushman, Antia, Udhayakumar, & de Roode, 2018). Genetic diversity can provide information on the current drive in drug resistance and help identify control measures based on epidemiological entities. Thus, selectively targeted interventions can be implemented in each distinct ecological zone (Adjah et al., 2018). As a result, the purpose of this study was to ascertain the genetic characteristics of *P. falciparum* in Ghana's Central Region.

CHAPTER THREE

METHODOLOGY

Design and conceptual framework of the study

Cross-sectional study was employed in this study with two domains. The first part was a parasitological survey conducted on suspected and confirmed *Plasmodium*-infected participants from August 2020 – June 2021. In five (5) study locations in the Central Region of Ghana, the prevalence of *Plasmodium* infections and risk factors were examined. The second phase consisted of analysing the genetic diversity of *P. falciparum* parasites and providing details regarding the complexity of infection within each ecological zone and season. Finally, it examined SNPs in validated markers of drug resistance of the *P. falciparum kelch*-domain protein (*Pfkelch13*), Chloroquine resistance transporter (*Pfcrt*), multidrug resistance protein-1 (*Pfmdr1*), *Pfdhps and Pfdhfr* genes, using Sanger sequencing approach. The Sequencing of *P. falciparum* strains was carried out in the Malaria Genome Laboratory at the Wellcome Sanger Institute, Hinxton UK under a collaborative agreement with Dr. Enoch Aninagyei (1241-PF-GH-ANINAGYEI), University of Health and Allied Sciences, Ho. The study's conceptual framework is depicted in Figure 6.

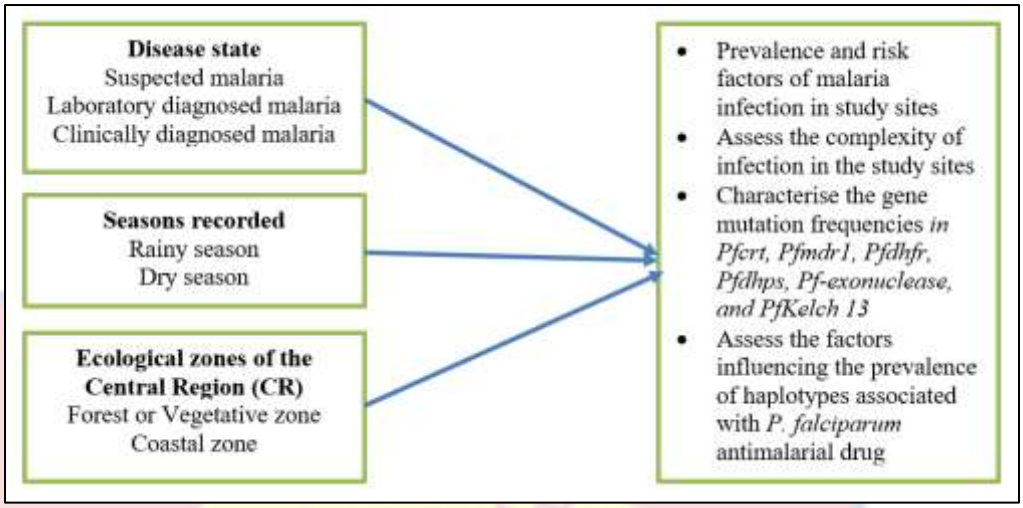


Figure 6: The study's conceptual framework

Ethical clearance and participants' informed consent

This study was performed following Good Clinical Practices, whilst observing the national COVID-19 protocols. The Central Regional Health Directorate's Ethics Committee (CR/G-81/507) and Ghana's Ghana Health Service Ethics Review Committee (GHS-ERC) (GHS-ERC Number: GHSERC017/03/20) approved the study. In the case of children under the age of 18 years, written informed consent was obtained from their guardian or parent. Written informed consent was also sought from adults above 18 years.

The study region

Ghana's Central Region covers approximately 6.6% (9,826 square kilometers) of the country's total land area. It is surrounded to the west by the Western and Western North Regions and to the south by the Gulf of Guinea. Additionally, the region borders the Greater Accra Region on the East, the Ashanti Region on the North, and the Eastern on the North-East. The historic city of Cape Coast serves as the capital of one of the region's 22 administrative districts. Rural areas make up 63% of the region. The primary occupations are fishing and farming on subsistence level (RHD annual report, 2018). The

actual population of the region is 2,605,490. The region is postulated to grow at a rate of 3.1% whilst about 215 inhabitants occupy a square kilometer (RHD annual report, 2018). Transmission of malaria is ongoing, with the most peaks occurring during the months of March through June and October through November. Malaria is mostly caused by *P. falciparum* species in the region.

Ecological features of the region

The region enjoys a major (April to July, peaking in June) and minor (September to November, peaks in October) rainy season per year. The vegetation cover is divided into coastal savannah (about 15 km inland), stretching parallel to the Gulf of Guinea, and a tropical rain forest covering inland areas towards its Ashanti and Eastern regional borders. The Kakum National Park is a forest reserve in the Twifo Hemang Lower Denkyira district, and its about 25 kilometers from Cape Coast. The average temperature ranges from 21–31°C whilst the annual rainfall ranges from 750–1000 mm (Tormey & O'brien, 1993).

Districts located in the Central Region area

There are 13 districts in the vegetation/forest zones and nine in the coastal zone (See Appendix 6)

Selection of study districts

Study sites were selected in 2 stages. Optimum allocation approach was used to select, three districts randomly from the forest zone, and two districts from the coastal zone. First, the names of all 22 districts were written on equal-sized pieces of paper and neatly folded such that the names were not visible on the outside. The folded papers were categorised into two groups, the forest zone, and the coastal zone, placed in different transparent wide-mouth

plastic containers with corresponding labels and tightly covered with lids. The containers were shaken severally to achieve an even mix. An independent person picked three of the folded papers from the forest category and two from the coastal category.

Selection of study health facilities within study districts

From each study district, the names of all the public health referral facilities were placed in a transparent wide-mouth plastic container. Similarly, an independent person randomly selected the study health facilities. Five health facilities were selected. They were Abura Asebu Kwamankese (Abura Dunkwa District Hospital) and Cape Coast Metropolis (Ewim Polyclinic) for Coastal districts, while Gomoa East District (St. Gregory catholic hospital), Assin North Municipality (Saint Francis Xavier Catholic hospital) and Agona West district (Agona Swedru municipal hospital) were selected for forest districts.

Ewim Polyclinic is a public health facility in the Cape Coast Metropolis. It was established in 1976, with a bed capacity of 25, serving an estimated population of 40,955. In 2019, malaria accounted for 18% of the total diseases recorded at the facility. In 2019, the facility tested 20,384 with about 5000 positive cases (Unpublished data).

Abura Dunkwa District Hospital located in Abura Dunkwa was established in 1946 as a clinic and later upgraded to a district hospital in the year 2000. It also serves as a referral facility for the district. The facility is a 56-bed capacity hospital that sees about 5000 suspected malaria cases and reports about 1000 positive cases yearly.

Saint Gregory Catholic Hospital is one of the Roman Catholic Mission facilities located in Budumburam in the Gomoa East district in the Region. It was established in 2005. It provides 24-hour health care services to the people of Kasoa and its environs. The hospital performs about 30,000 malaria testing, with 5000 positive cases recorded annually (Unpublished data).

Saint Francis Xavier hospital is also a Roman Catholic Mission facility in the Assin Central Municipality. It was established in 1965 by the Catholic Archdiocese of Cape Coast, with a bed capacity of 145. In 2019, the facility reported 1,375 malaria-positive cases, out of about 3000 cases tested. It serves as a referral facility to both Assin North and South. It is also a training facility for various health education institutions within and outside Ghana.

Agona Swedru Municipal hospital is a 162-bed capacity hospital in the Agona West district. The hospital provides 24-hours service and conducts about 27,000 malaria cases with about 9,000 positive cases annually (unpublished data). Figure 7 represents the geographical locations of the five (5) study sites selected in the region.

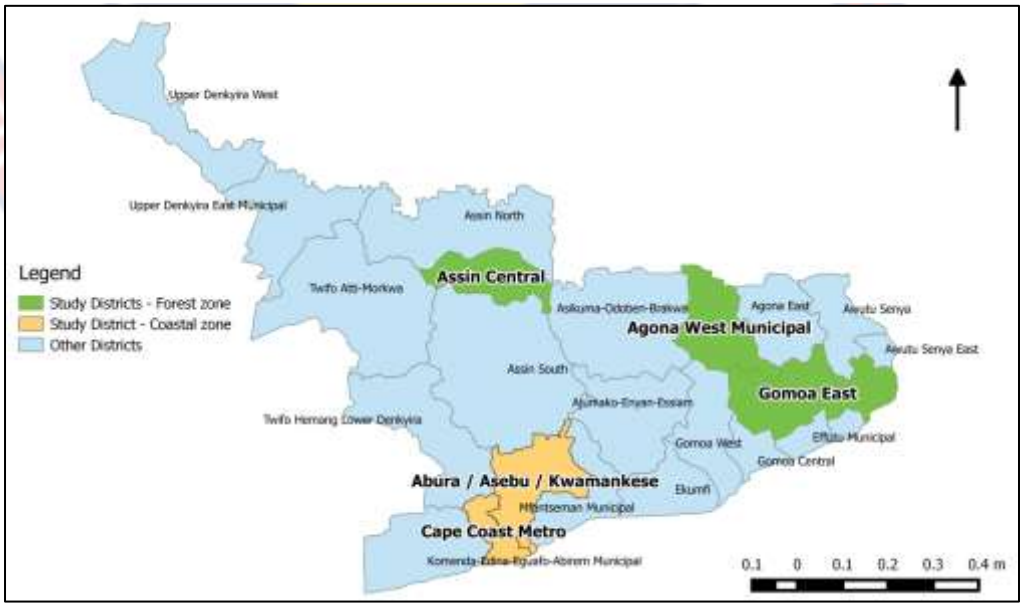


Figure 7: Map of Central Region indicting selected study districts in the forest (Green) and coastal (Orange) zones and other districts (Blue).

Sample size calculation

According to the Ghana Health Service's 2016 annual report, 39.0% of OPD cases in the Central Region were caused by malaria (GSS, 2017). Table 2 below shows the population of selected study districts as per the 2010 census.

Table 2: The selected districts and their population for 2010

District	Population (2010 Census)
Abura Asebu Kwamankese	151,185
Assin central Municipality	161,341 (Assin South was used as a proxy)
Gomoa East District	195,306
Cape Coast Metropolis	169,894 (Cape Coast Metropolis was used as a proxy)
Agona Swedru Municipality	115,358

Using the formula, $n = \frac{N}{[1 + \mathrm{N}\ (\mathrm{e})^2]}$

The minimum sample size for each study district was determined using the various populations in each district, according to the 2010 population census. n is the sample size, N is the finite population, and e is the limit of tolerable error (0.05). However, the population in Gomoa East District was used to approximate the number of samples to be collected from each district as the Gomoa East District is the most populated. As a result, at least 399 study participants were recruited from each study district. Hence, the minimum number of suspected malaria cases sampled from each study district was 399.

Study population

In the selected health facilities, both suspected and confirmed malaria cases were the focus of the study.

Conditions for participant inclusion

Participants involved in this study were permanent residents of each study district. Also, participants who consented to be a part of the study, participants suspected of malaria by a clinician, participants who were able to provide study information either by themselves or by their relatives, and who provided the required volume of blood for analysis were included in the study.

Conditions for participant exclusion

Participants who met the inclusion criteria but were unable to provide study information either by themselves or through their relatives were excluded. Participants who could not provide the required volume of blood for analysis were also excluded. Also, Individuals were excluded from the study if they were not permanent residents in the selected districts or they if participants opted out of this study.

Sensitization of health professionals regarding the study and training of research assistants

A series of meetings were held with the Management of each health facility, the prescribers/clinicians, and the personnel of the laboratory department, regarding the study. Research Assistants in persons of Certified Medical Laboratory Scientists, were selected at each study site and adequately trained on blood sample collection and storage. They were also trained on how to obtain consent from participants or their guardians, administer questionnaires and retrieve information from both participants' clinical and laboratory records. A refresher training was also given on how to prepare thick and thin blood smears. The sample collection for this study was carried out simultaneously in all the study sites through the trained research assistants.

Participant enrolment

Informed consent

Participants received all required information; in languages they understand very well. This included information about the study's procedures, purpose, compensation, benefits, and risks associated with participation, confidentiality, termination, and duration in accordance with international and local guidelines for human subject research. Participants were given ample time to consider enrolling in the study and ask questions. Participants or the parents or guardians of children under the age of 18 years who willingly decided to enrol in the study were asked to sign their informed consent.

Criteria for withdrawal from the study

Participants were required to inform the research assistants of their withdrawal from the study. All participants were at liberty to withdrawal from the study at their convenience.

Procedures for sample collection

Collecting blood samples from adults

For each RDT-positive patient, whole blood was collected from the antecubital fossa, by performing a venepuncture. The site and its surrounding skin surface were disinfected with 70% isopropyl alcohol. The venepuncture site was allowed to dry for a minimum of 30 seconds. From each adult study participant, 1.5 ml of whole blood samples were drawn and dispensed into 1.45ul of Citrate Phosphate Dextrose Adenine (CPDA) anticoagulants in 1.5ml Eppendorf tubes. Tubes were tightly close and stored at 2-8^{o}C for further analysis. This was used for the preparation of blood films for parasite detection, and quantification as well as Dry Blood Spots for genome studies.

The presence of the parasite in the patient sample was established by detecting histidine-rich protein 2 antigens using CareStart rapid diagnostic test kit (Access Bio, Somerset, USA), followed by visualisation of the parasites in thick and thin blood smears.

A questionnaire was designed and administered to elicit Patient demographic data. Data collected from the questionnaires included age, sex, educational level of patient/ guardian, premedication with an antimalarial agent and malaria risk indicators, and control practices information such as the use of long-lasting insecticide-treated net usage.

Collecting blood samples from children

For children 3-5years who tested positive during RDT testing, a volume of at least 1 mL of venous blood was collected and dispensed into 1.45ul of Citrate Phosphate Dextrose Adenine (CPDA) anticoagulants in a 1.5ml Eppendorf tube. For children 0-2 years, free-flowing capillary blood samples of about 300ul were collected into the capillary tubes. Tubes were tightly closed and stored at 2-8^{o}C for further analysis.

Safety Considerations employed for sample collection:

Specimens were collected with adherence to the National directives on Social Distancing and the use of Personal Protective Equipment (PPEs), taking into consideration Covid-19 pandemic prevention protocols. All participants were attended to, with a fresh pair of gloves, a new sterile lancet or syringe, and a needle. Participants were given maximum privacy when filling in the questionnaire. Questionnaires and blood preparations were coded so that they cannot be traced back to the Participants.

Collection of study information

The laboratory registers and selected hospitals' Health Administration and Management Systems (HAMs) were the sources of participant demographic and clinical data. A list of participants suspected of malaria, and sent to the laboratory was collated from the laboratory register. The data collected included the participants' hospital ID, age, sex, request for a malaria test, results of malaria tests conducted, and all other tests requested for the same patient. This made the list of total suspected and confirmed malaria cases. The participant's hospital ID number was searched in HAMs to confirm the details collected from the laboratory register (where applicable). All patient information retrieved was keyed into excel. Participants' names were coded such that they could not be traced back to the participants. Study participants' demographic information was collected in both rainy (September to November 2020) and dry seasons (December 2020 to February 2021) in the forest and coastal areas.

Sample transportation

Blood samples and accompanying documentation were retrieved from the study sites every two weeks and transported on ice to the laboratory unit of Ewim Polyclinic in Cape Coast. The estimation of Parasite density and preparation of dried blood spots were carried out at the Polyclinic.

Laboratory procedures

All samples collected from the study sites were screened with both rapid immunochromatographic tests and microscopy. Triplicates of thin and thick blood smears were prepared per sample according to the standard protocol. All samples were stained with Giemsa, air-dried, and observed under

a light microscope. Dried blood spots were prepared for selective whole genome sequencing (sWGA), using Hiseq 2500. The workflow employed in achieving the second, third, and fourth objectives is outlined in figure 8.

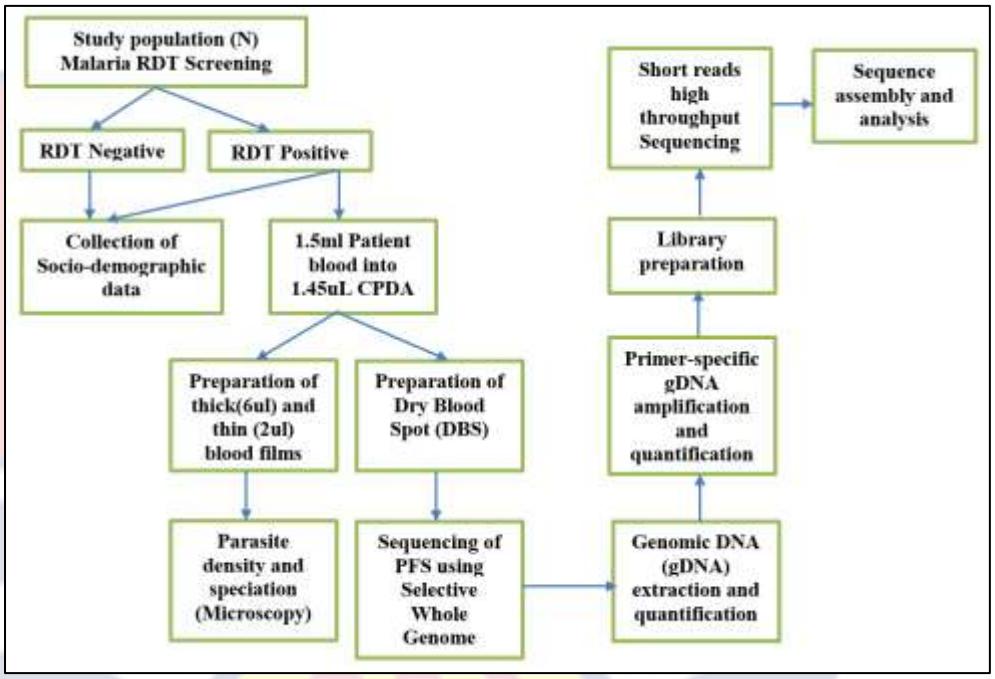


Figure 8: Conceptual Framework-Showing the pattern of workflow employed in the study

Malaria rapid diagnostic testing

Immunochromatographic screening of *Plasmodium* infections was done for participants with fever visiting the Outpatients and Antenatal clinics, using the rapid diagnostic tests (CareStart^{TM} Malaria HRP2/pLDH (*Pf*/Pan) Combo). The tests were carried out according to the manufacturer's instructions. Before the test kit was opened, the date of expiry was checked. The test kit was then checked for defects (cracks, colour change in cellulose paper). The kit was labelled with the patient Identification number. The middle finger of participants is disinfected with 70% alcohol. A lancet is used to prick the disinfected site. A full drop of blood (about 5 uL) was placed onto the cellulose paper in the RDT device, using the loop supplied by the manufacturer in the RDT package. Four (4) drops of buffer were added to the

buffer window. Results were interpreted after 15 minutes, and the positive result was confirmed with the presence of both the control and test lines on the device.

Malaria microscopy and parasite counting

Thick and thin blood films were prepared on a single slide per sample, with 6 µL and 2 µL of whole blood respectively. Three slides were prepared for each participant. The smears were air-dried and stained with 10% Giemsa (10 mL of Giemsa stock in 90 mL of phosphate-buffered saline) for 30 minutes. After staining, the slides were air-dried. Asexual parasites were observed at 1000x magnification under a light microscope. *Plasmodium* species were identified using thin films, and parasite density was determined by counting the number of asexual *P. falciparum* parasites per 200 WBCs using thick films. The resultant figure was multiplied by 8000 (assumed white blood cell density) per microliter (uL) according to the WHO protocol in the formula:

$$\text{Parasite density / uL} = \frac{\text{Number of parasites counted}}{\text{Number of leukocytes counted (200)}} \times 8000 \text{ uL}$$

Examinations were extended to 500 white blood cells in locations where fewer than ten parasites were found. If no asexual stage of the parasites was observed in 200 high-power fields, blood slides were deemed negative.

Quality Control

Apart from the trained staff of the study sites and the PI, a National Malaria Control Programme-certified microscopist reviewed all slides at Ewim Polyclinic. His opinion overrode all others. Figures 9,10, and 11 below show samples of stained blood smears.

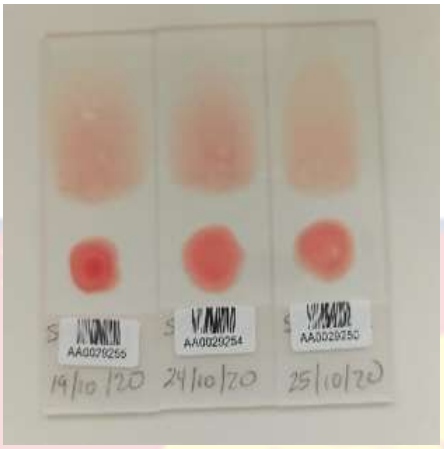


Figure 10: An unstained blood film Fieldwork 2020

Figure 9: Giemsa stained blood film Fieldwork 2020

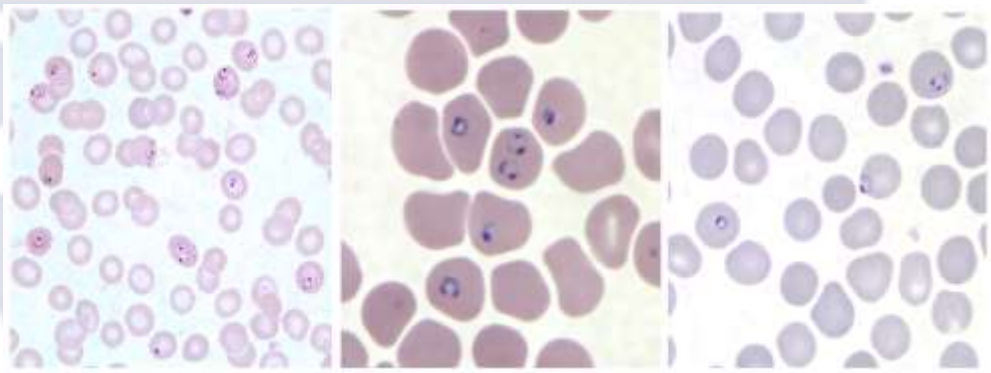


Figure 11: P. falciparum trophozoites in the stained thin blood film. Source: Fieldwork 2020

Criteria for selecting blood samples for preparation of dried blood spots

Blood samples of the first and fifth malaria-positive slides from each facility were retrieved for the preparation of the dried blood spots. Only blood samples showing positive tests for both RDT and Microscopy were selected, whilst RDT positive with undetectable asexual parasites during microscopy were excluded.

Preparation of DBS and associated metadata

Dry blood spots were prepared for microscopically confirmed blood specimens containing *P. falciparum* parasites, using materials as indicated in figure 12 below.

Figure 12: DBS preparation kit

Approximately 50µL of blood samples were collected on filter papers (3MMWhatman, United Kingdom). This made a 10 mm diameter spot. Two spots were made on each strip of filter paper. A total of four blood spots were made per sample, two on each paper card. One sample barcode was stuck to each paper card, with cards of the same patient bearing the same barcodes. Each sample barcode was specific to a single patient. The filter papers were air-dried at room temperature.

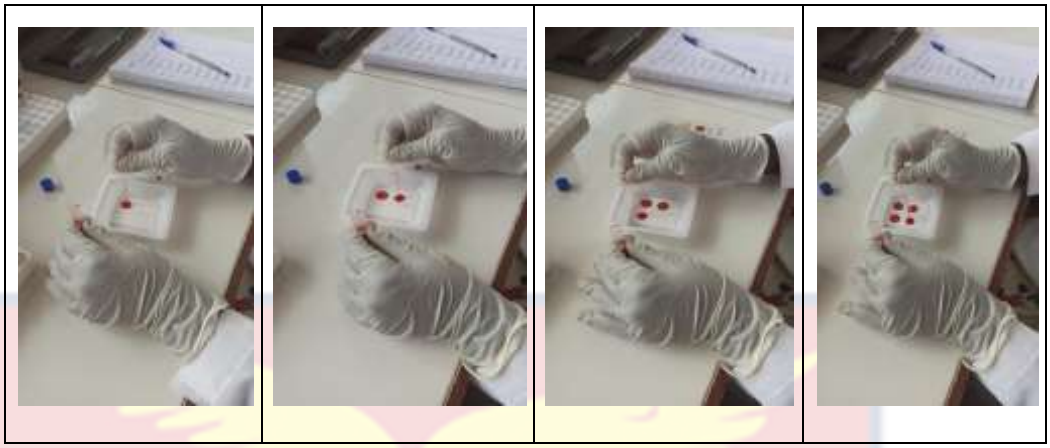


Figure 13: Pictorial description of the preparation of dry blood spots
Source: Fieldwork, 2021

Figure 14: Pictorial description of preparations per sample labelled with barcodes
Source: Fieldwork, 2021

Packaging and shipping of DBS

The pair of dry blood spots were packaged in plastic envelopes (zip lock bags) with a desiccant. These individual zip lock bags containing the patient specimen were also packed into a bigger plastic pouch, and finally into a bigger biohazard bag. All DBS were stored at room temperature (<25ºC) and shipped to Wellcome Sanger Institute, UK for molecular analyses. A third barcode was stuck on the sample information sheet that accompanied the samples to the Sanger Genome Centre, UK. Figure 15 shows the packing process before shipping of the DBS

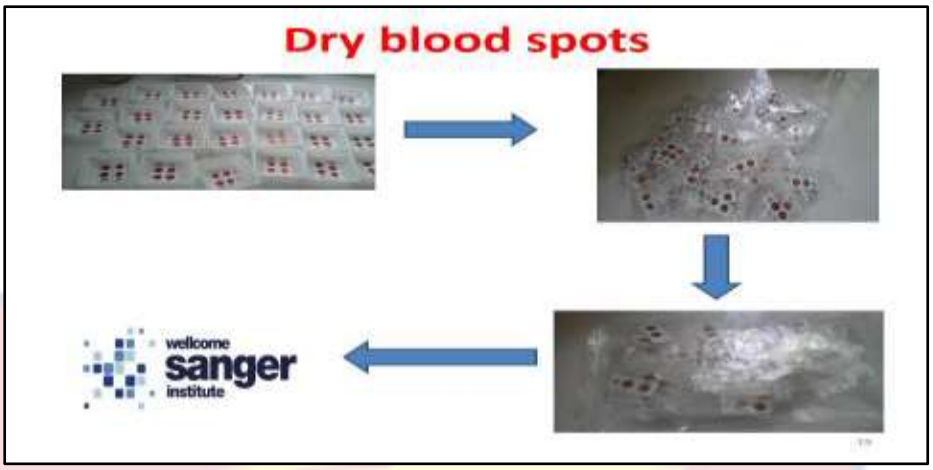


Figure 15: Packaging of DBS before shipping

Genomic DNA extraction from DBS

Extraction of *Plasmodium* genomic DNA (gDNA) using Automated DNA extraction

This is an extraction process to release the DNA of *P. falciparum* present in the DBS from filter paper blood spots using a QIAampDNA Investigator Kit (Qiagen, Germany), Dry blood spots (samples collected from study sites) were cut into small pieces of 3 mm diameter into an Eppendorf ™ Deepwell™ plate of 96 wells (Fisher Scientific, UK), using BDS 600PLUS robotic puncher (Microelectronic System, Brendale, Australia). About 6-8 punched DBS pieces punched from each sample were placed into approximately 2 ml deep microcentrifuge wells, and filled with 1 mL lysis buffer (1.4mL Buffer ATL and 100µl proteinase K) (Qiagen, UK). The samples were vortexed thoroughly to help the blood come free from the card. The plate containing the samples was placed on a themo-shaker (TS-100, Themo-Shaker, Latvia) set at medium speed, and allowed to mix overnight. The resulting haemolysate after the tubes have spun overnight was transferred to a 96-well filter plate (OmegaBio-tek, Austria) and sent to QIAcube HT Qiagen for automated DNA extraction and purification.

In the Qiacube automated system, 300µL Buffer AL is automatically aspirated and added to each well. The wells were heated and incubated at a temperature of 70 °C on a themo-shaker set at 900 rpm for 10 minutes. Afterward, 150µl absolute ethanol was added into the wells and mixed with a vortex thoroughly. The samples were then transferred into a binding column. Using vacuum pressure, the mixture (haemolysate, Buffer AL, and absolute ethanol) was filtered into another 96-well plate through a binding column (a plate containing a silica-embedded filter paper. They are also termed filter plates). The binding column selectively binds to DNA and RNA, attaching the nucleic acids to its silica gel content whiles allowing the filtration of cellular proteins and polysaccharides into a collection well. The extract is purified by washing it with 500µl wash Buffer (AW1 and AW2) sequentially to remove residual protein contaminants and some amount of salts. After the washing process, the 96-plate binding column was placed on another 96-well plate, and 700µl of absolute ethanol was dispensed into the wells to help remove residual salts during vacuum filtration. Another vacuum filtration is carried out without ethanol to dry out the binding column. The binding column was then placed on a DNAse/RNAse free 96-well and 20–100µl elution Buffer ATE dispensed directly into the middle of the filter in the binding column. This was incubated for 5 minutes and the elution buffer was forced through the filter into the new wells. The pure nucleic acid extract is kept on ice and a portion is pipetted. The processes are summarized in Figure 16. The extracted gDNA is quantified using QuantiFluor on Jenway 737-501 analyser to estimate the concentration of DNA yield and its suitability for amplification and sequencing.

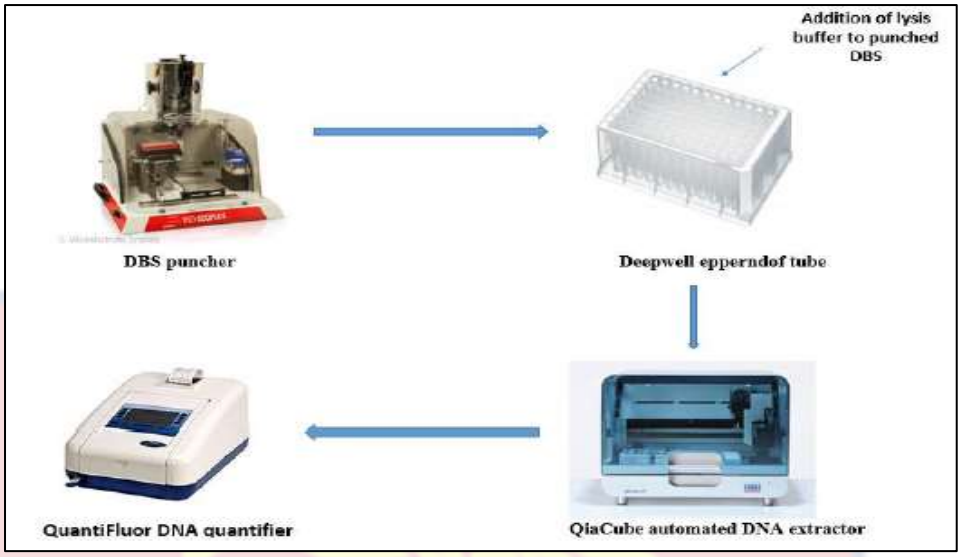


Figure 16: Direction of flow of gDNA extraction and quantification

Selective whole genome amplicon sequencing (sWGA) assay

Selective whole genome amplicon sequencing (sWGA) was used to detect and speciate *Plasmodium* parasites in dry blood spots. The methodology has been previously described (Aninagyei et al., 2019). *Plasmodium* DNA in DBS samples was extracted using QIAamp DNA Investigator Kit (Qiagen, Germany). Previously, BDS 600PLUS robotic puncher (Microelectronic System, Brendale, Australia) punched 6-8 DBS into the Eppendorf^{TM} Deepwell^{TM} plate of 96 wells (Fisher Scientific, UK). DNA (human and *Plasmodium*) were isolated using a Qiagen DNA extraction kit following the manufacturing protocols. The sWGA reaction was performed in 0.2 mL PCR plates. The total volume of the reaction was 50µL. It contained a minimum of 5 ng of template DNA, 1× BSA (New England Biolabs), 1 mM dNTPs (New England Biolabs), 2.5µM of each amplification primer, 1× Phi29 reaction buffer (New England Biolabs), and 30 units of Phi29 polymerase (New England Biolabs). The reaction plates were placed in an MJ thermal Cycler (Bio-Rad, UK) programmed to run a "stepdown" protocol consisting of 35 °C for 5 min, 34 °C for 10 min, 33 °C for 15 min, 32 °C for 20 min, 31 °C for 30

min, 30 °C for 16 hours then heating at 65 °C for 15 min to inactivate the enzymes before cooling to 4 °C. Here, all *Plasmodium* DNA present were amplified, but not human DNA. *Plasmodium* amplified products were quantified using Qubit® dsDNA high sensitivity (Thermo Fisher Scientific, UK) to make sure a minimum of 500 ng DNA was sent for sequencing. Preceding sequencing, sWGA products were cleaned using Ampure XP beads (Beckman Coulter, UK) as recommended by the manufacturer. Briefly, 1.8 volumes of beads per 1 volume of the sample were mixed and incubated for 5 min at room temperature. After incubation, the tube containing the bead/DNA mixture was placed on a magnetic rack to capture the DNA-bound beads while the unbound solution was discarded. Beads were washed twice with 200 µL of 80% ethanol and the bound DNA was eluted with 60 µL of elution buffer. Cleaned amplified DNA products of about 0.5–1 µg DNA were used to prepare the DNA library using the NEBNext DNA sample preparation kit (New England Biolabs). DNA libraries were sequenced using Illumina HiSeq 2500 DNA sequencer.

Multi-deplexing of sequences

The outcome of the HiSeq sequencing was sequences of multiple samples pooled together. An index tag (also called a barcode) consisting of a unique sequence of about 6 and 12bp was added to each sample so that the sequence reads from different samples could be identified. On the Illumina HiSeq, the process of demultiplexing (dividing obtained sequence reads into separate files based on each index tag/sample) and generating the FASTQ data files was done for downstream analysis, carried out automatically using the on-board PC preinstalled with FASTQ data readable software. So, at this

point, each sample was deplexed into individual barcode 101 bases. These bases represent the conserved region on each gene detected. Undetected and heterogeneous genes were designated 'X' and 'N' respectively. Figure 17 shows the bioinformatic pipeline for the analysis of Sequenced *Plasmodium* genes.

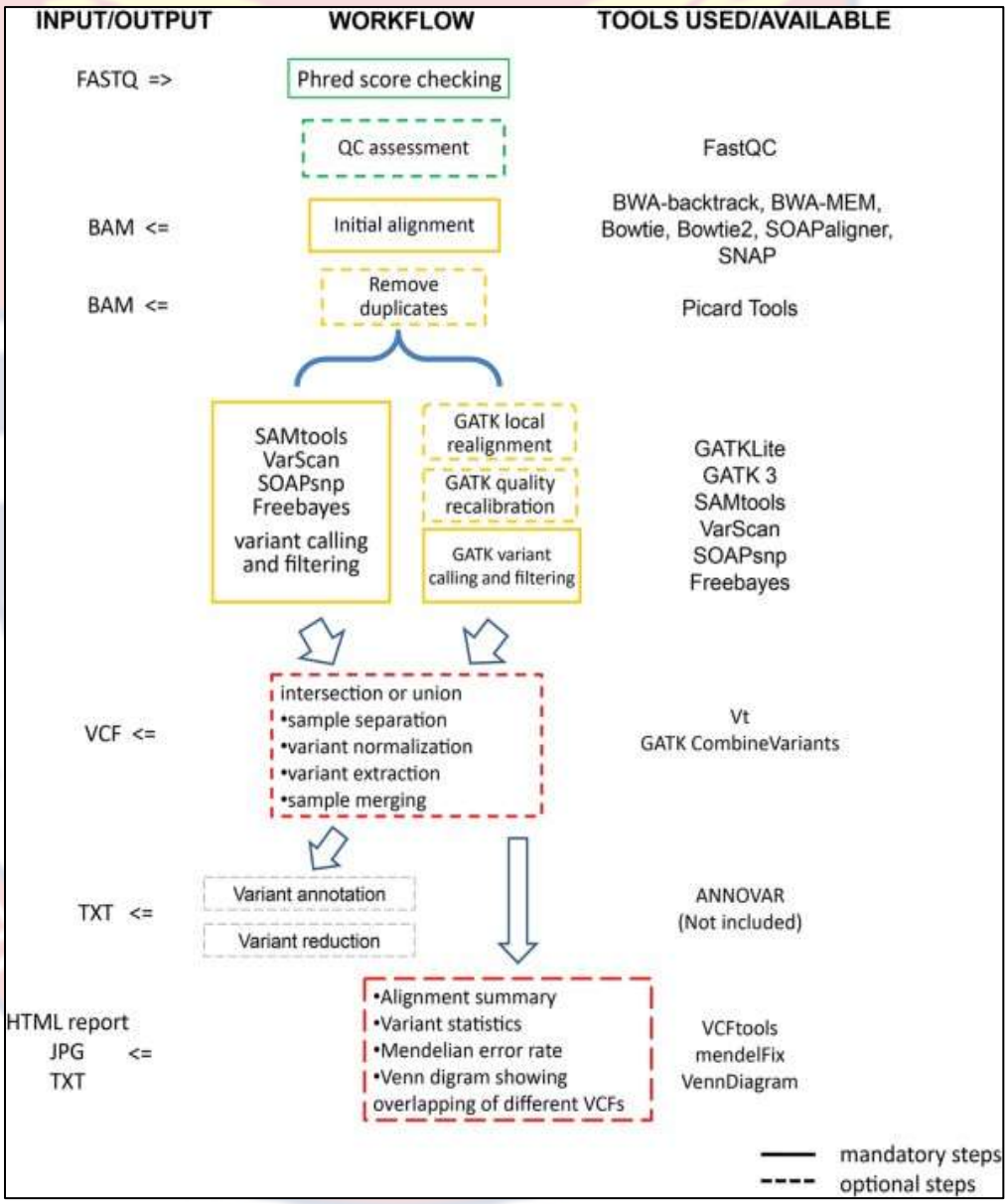


Figure 17: Shows bioinformatic pipeline for the analysis of Sequenced *Plasmodium* genes.
Source:(Guo, Ding, Shen, Lyon, & Wang, 2015)

Objective 2: Genomic characterization of *P. falciparum* COI/MOI using McCOIL

Determination of COI

All samples collected from the study sites were screened with both rapid immunochromatographic tests and microscopy. In accordance with the standard procedure, thin and thick blood smears were prepared for each sample on a glass slide. The samples were stained with 10% Giemsa, air-dried, and observed under a light microscope. Sorting was done on the samples that tested positive for *Plasmodium* infection and Dried blood spots were prepared for selective whole genome sequencing (sWGA) using Hiseq 2500. The workflow employed in achieving objectives 2,3 and 4 is outlined in figure 8.

Methodology

SNPs were determined by sWGA, with reference to the short sequences designed to identify conserved regions of *P. falciparum, P. ovale, P. malariae* and *P. vivax.* A sample was determined to contain a single or mixed species or multiple clones of parasites after a multiple alignment programme was run using Multiple Alignment using Fast Fourier Transform (MAFFT) software. SNPs were identified by examination of the respective amino acid positions for wild-type amino acids. Any mutations identified were compared with established mutations associated with various anti-malaria drugs. The complexity of infection (COI) was determined using the average heterogeneous calls (two or more alleles found on the same amino acid position) for the parasite strain to estimate COI, using COI likelihood based on Bayesian approach (Galinsky et al., 2015), and finally, *P. falciparum* strains

heterogeneity was determined by comparing the barcode of selected gene positions.

Objective 3: Genomic characterization of *P. falciparum* in determining SNPs associated with drug resistance

A total of 522 *P. falciparum* parasite strains were sequenced using sWGA for SNPs in the *Pfcrt, Pfdhfr, Pfdhps, Pfmdr1, Pf-exonuclease,* and *Pf kelch 13* genes. The workflow employed in achieving this objective is outlined in figure 8.

Data handling, statistical analysis, and analysis of genomic data

Analysis of baseline malaria data

Baseline malaria data were collected every two weeks and entered into Microsoft Excel 2019. Microsoft Excel 2019 data was imported and converted into STATA Version 15 (StataCorp LLC, Texas - USA) for statistical analysis. For categorical variables (that is, age group, gender, patient type, study sites, ecological zones, seasons, and malaria status), descriptive analysis was conducted using frequencies and percentages. The outcome of interest was participants with malaria. The percentage of participants who tested positive for malaria was calculated by dividing the total number of cases by the total number of participants who tested for malaria multiplied by 100.

Test of association was conducted using Pearson chi-squared test between independent variables [demographic characteristics (i.e. age, gender, patient type, and study sites), environmental factors (i.e. ecological zones – forest/coastal and seasons – dry/rainy)] and dependent variable [participants with malaria]. Further statistical test by stratification was carried out to determine if any independent variable would significantly associate with the

dependent variable when the former shows no significant statistical relationship. Simple and multiple logistic analyses were also carried out to assess the influence of independent variables on the outcome of interest (dependent variable). P-values of less than or equal to 0.05 were considered statistically significant in the inferential statistics.

Analysis of genomic data

Successfully sequenced data obtained from each of the 409 samples were analysed. In the analysis of the genomic data, all parasite strains that were not fully sequenced were not analysed. Descriptive analysis was done using frequencies and percentages for categorical variables such as age group, gender, patient type, study sites, ecological zones, and seasons. The data were described using frequency distributions, means, and standard deviations. To achieve a test of normality, Shapiro-Wilk and Bartlett's test was used to assess the symmetry of all continuous data. The distribution of drug resistance strains was compared based on propositions of complete parasite sequences that contained drug-resistant gene mutations. The mean complexity of infections was determined for each study area, and comparative barcodes were done manually by aligning each locus and determining the frequency of heterogeneity. The complexity of infection (COI) was determined for every sample as described by Galinsky (Galinsky et al., 2015), with little modification. The program estimates COI from bi-allelic SNP genotyping data using the binomial distribution, which is based on: 1) each SNP locus' population minor allele frequency (MAF) and 2) the number of polymorphic genotypes in each 101-SNP barcode (Galinsky et al., 2015). COIL was run with default settings (COIL Estimator. https://github.com/COIL2/COIL2) (i.e.,

genotyping error rate of 5% and a uniform prior), except for the MAFs, which were manually calculated at each SNP position. From the binomial-SNP barcode, COIL can distinguish between up to 22 levels. Using the Burrows-Wheeler Aligner, the sequence data from each sample was independently mapped to the 3D7 reference genome using standard Illumina Quality Control procedures (Oyola et al., 2016). Before that, raw reads were de-multiplexed and filtered using parameters by Torrent Suite pipeline software. Read quality was assessed using the Torrent Suite FastQC plugin v0.10.1, before high-quality reads were aligned to the reference genome. Each amplicon target gene's 300 bp flanking regions and complete gene sequence were included in the reference data file, which was a multi-FASTA file from the *P. falciparum* 3D7 genome (Bahl et al., 2003). Two independent samples t-test was also performed to test for differences in mean Complexity of infection (COI) stratified by independent variables (gender, ecological zones and seasons). One-way analysis of variance (ANOVA) between COI and age group, study sites, and mean parasite density.

Proportions of mutations in *Pfcrt, Pfdhfr, Pfdhps, Pfmdr1, Pfexonuclease, and Pf kelch-13* genes in the study districts were also calculated using frequencies and percentages. Pearson chi-squared test of association between independent variables [demographic characteristics (i.e. age, gender, patient type, and study sites), environmental factors (i.e. ecological zones – forest/coastal and seasons – dry/rainy)] and dependent variable [mutations in *Pfcrt, Pfdhfr, Pfdhps, Pfmdr1, Pf-exonuclease, Pf kelch-13* genes] were also determined. In order to explore Haplotypes associated with *P. falciparum* antimalarial drug resistance, six different models were

conducted using multiple logistic regression analysis. Model I looked at how demographic and environmental factors affect *Pfkelch13* resistance in *Pf kelch13*. Model II investigated at how demographic and environmental factors affect putative drug-resistant haplotype to the *Pfcrt* gene. Model III also investigated how demographic and environmental factors affect the Putative Pyrimethamine Resistant Haplotype in the *Pfdhfr* gene. Model IV also investigated how demographic and environmental factors affect putative Sulfadoxine resistant haplotype in *Pfdhps*. Model V assessed the effect of demographic and environmental factors on putative drug-resistant haplotype in the *Pfmdr* 1 gene. Finally, Model VI investigated the effect of demographic and environmental factors on putative drug-resistant haplotype in the combined *Pfdhfr/Pfdhps* gene mutations using multiple logistics regressions. The successful sequence data was exported and organised in Microsoft Excel 2019. For statistical analysis, the data were imported and converted into STATA version 15 (StataCorp LLC, Texas, USA). All analyses were performed using STATA version 15. *P*-values below 0.05 were considered statistically significant.

CHAPTER FOUR

RESULTS AND DISCUSSION

RESULTS

OBJECTIVE 1: To investigate the prevalence and risk factors of malaria among patients in the Central Region

Total patient enrolees and distribution by district, seasons, and ecological zones

The study spanned from August 2020 – June 2021 and captured in total, 3993 participants suspected of malaria from the five study districts. Of the 3993 study participants, 529 (13.3%), 660 (16.5%), 729 (18.3%), 872 (21.8%), and 1203 (30.1%) participants were selected from Abura-Asebu-Kwamankese District Hospital in the Abura-Asebu-Kwamankese District (AAK), Agona Swedru Municipal Hospital in the Agona Swedru Municipality (ASM), Ewim Polyclinic (EPC) in the Cape Coast Metropolis (CCM), Saint Gregory Catholic Hospital (SGCH) in the Gomoa East District (GED) and Saint Francis Xavier hospital (SFXH) in the Assin Central Municipality (ACM), respectively. Samples were categorized into seasons of collection (rainy and dry) as well as ecological zones (Forest and Coastal zones). The rainy season contributed 3029 (75.86%) of the total samples whilst the dry season contributed 964 (24.14%). From the ecological zones, study sites in the forest zones contributed a total of 2735 (68.49%) samples, whilst the coastal zones contributed 1258 (24.14%). The study shows a significant relationship between malaria prevalence and Seasons with *P*-value<0.001. There was also significant relationship between malaria prevalence and Ecology with *P*-

value=0.007. The study shows a significant relationship between malaria prevalence and study districts with *P*-value=0.001 (Table 3).

Table 3: Distribution of participants by district, seasons, and ecological zones

Characteristics	Number of participants [N=3993 (%)]	Number of participants with RDT suspected malaria [2495 (61.81) N (%)	Number of participants with microscopy-confirmed parasitaemia [1525 (61.1)] n (%)	*P*-value
Season				<0.001**
Rainy	3029 (75.86)	1826 (73.19)	1089 (71.41)	
Dry	964 (24.14)	669 (26.81)	436 (28.59)	
Ecology				0.007*
Forest zone	2735 (68.49)	1819 (72.91)	1083 (71.02)	
Coastal zone	1258 (31.51)	676 (27.09)	442 (28.98)	
Study district				0.001**
ACM	1203 (30.13)	676 (27.09)	393 (25.77)	
GED	872 (21.84)	530 (21.24)	330 (21.64)	
AAK	529 (13.25)	182 (7.29)	106 (6.95)	
CCM	729 (18.26)	494 (19.80)	336 (22.03)	
ASM	660 (16.53)	613 (24.57)	360 (23.61)	

Source: Field Work 2021: Total participants suspected of having malaria-3993
*p<0.05 and **p<0.001 were said to be statistically significant. AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality

Demographic characteristics and parasitological outcome of the participants suspected of malaria

Participants in the outpatient department accounted majority of 1918 (48.03%) of the study participants suspected of malaria, followed by in-patients 1196 (29.95%), while ante-natal attendants 879 (22.01%) were in the minority. The majority of the participants, 2759 (69.10%), were between the ages of 20-59 years. Children under 5 years were 385 (9.62%), whilst those above 60 years accounted for 407 (10.19%). Malaria rapid diagnostic test kit (RDT) yielded 2495 (61.81%) positive cases, out of which 1525 (61.1%) were

also positive by microscopy. However, this places prevalence of microscopy confirmed malaria among the 3,993 recruited patients at 38.19%. The Samples from female participants 3040 (76.13%), participants seen at the outpatient department 1918 (48.03%), samples collected during the rainy season 3029 (75.86%), and from the forest zone 2735 (68.49%) dominated in their respective categories (Table 4).

Table 4: Demographic characteristics of participants

Characteristics	Number of participants [N=3993 (%)]	Number of participants with RDT suspected malaria [2495 (61.81)] n (%)	Number of participants with microscopy-confirmed parasitaemia [1525 (38.2)] n (%)	*P*-value
Age group (years) [Mean (SD)]	31.80±19.86			0.014*
Below 5	384 (9.62)	272 (10.90)	160 (10.49)	
5 – 9	120 (3.01)	90 (3.61)	59 (3.87)	
10 – 14	79 (1.98)	52 (2.08)	29(1.90)	
15 – 19	244 (6.11)	157 (6.29)	104 (6.82)	
20 – 59	2759 (69.10)	1689 (67.70)	1036 (67.93)	
60 plus	407 (10.19)	235 (9.42)	137 (8.98)	
Gender				0.539
Male	953 (23.87)	609 (24.41)	372 (24.34)	
Female	3040 (76.13)	1886 (75.59)	1153 (75.61)	
Patient type				<0.001**
OPD	1918 (48.03)	1144 (45.85)	690 (45.25)	
Inpatient	1196 (29.95)	636 (25.49)	379 (24.85)	
ANC	879 (22.01)	715 (28.66)	456 (29.90)	

Source: Field Work 2021: Total participants suspected of having malaria-3993
Total patient RDT positive-2495 (61.81%)
Total patient RDT negative-1498 (38.19%)
Total patients that were microscopy confirmed out of the positive RDT-1525(61.1%)
Total number that was microscopy negative out of the RDT positive - 970(38.9%)
*p<0.05 and **p<0.001 were said to be statistically significant.

District and gender stratification of study participants

Females contributed the largest number of participants in all the study districts, irrespective of ecological classification (Table 5). Females (n=633) in the Cape Coast metropolis were six (6) times more than their male (n=96) counterparts. In Abura-Asebu-Kwamankese District, female participants (n=431) were four times more than male participants (n=98). In Agona Swedru Municipality, Gomoa East District, and Assin Central Municipality the female enrollees were at least twice more than their male counterparts (Table 5).

Table 5: Gender prevalence of the study participants

		Number of participants		
Study Districts	**Ecological classification**	Male n (%)	Females n (%)	Total n (%)
AAK	Coastal	98 (18.5)	431 (81.5)	529 (13.2)
ASM	Forest	161 (24.4)	499 (75.6)	660 (16.5)
ACM	Forest	367 (30.5)	836 (69.5)	1203 (30.1)
GED	Forest	231 (26.5)	641(73.5)	872 (21.8)
CCM	Coastal	96 (13.2)	633 (86.8)	729 (18.3)
Total		953 (23.9)	3040 (76.1)	3993 (100)

Data are presented as numbers and percentages. Source: Field Work 2021
AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality

Age stratification of the study enrollees

In all, the age distribution of enrollees was 0 – 101 years. The highest mean age was observed in the Assin Central Municipality (34.5±24.7 years), whilst the least was recorded in Agona Swedru Municipality (29±14.8). The median age of participants ranged from 28 years recorded in Abura-Asebu-Kwamankese District to 30 years recorded in both Assin Central Municipality and the Cape Coast Metropolis as shown in Table 6.

Table 6: Age distribution of the study participants

	Age of participants in years				
District	Range	Mean±SD	Mode	Median	Q1 – Q3 (IQR)
AAK	86 (0 – 86)	30.4±19.4	20	28	20 – 38 (18)
ASM	101 (0 – 101)	29±14.8	30	29	22 – 34 (12)
ACM	99 (1 – 100)	34.5±24.7	1	30	19 – 45 (26)
GED	90 (0 – 90)	29±17.6	2	29	20 = 37 (17)
CCM	94 (2 – 96)	34.1±16.5	24	30	24 – 40 (16)

Source: Field Work 2021
Q1 - Lower quartile, Q3 - Upper quartile, IQR - Interquartile range, SD - Standard deviation. AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality

Distribution of study participants by type of service sought

In all the study sites, outpatient enrollees were in the majority whereas, in Abura-Asebu-Kwamankese District, Gomoa East District, and Assin Central Municipality, this was closely followed by in-patients, in Agona Swedru Municipality (n=225) and the Cape Coast Metropolis (n=202) recorded more antenatal patients than in-patients. In the Assin Central Municipality and the Gomoa East District, the out-patients were at least three times more than those in the ante-natal category, recording 590 outpatients compared to 185 ante-natal patients, and 517 outpatients compared to 145 ante-natal patients respectfully (Figure 18).

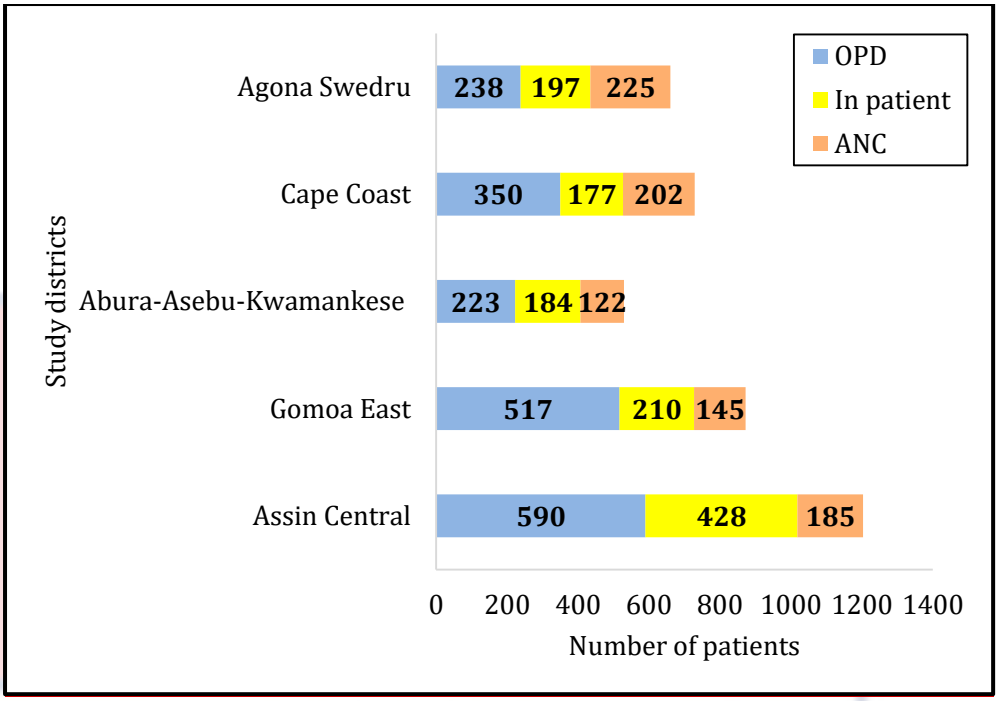


Figure 18: Distribution of patient types in the health facilities. There is a statistically significant association between study districts and patient types in the health facilities

Prevalence of malaria by study district

Out of the three thousand, nine hundred and ninety-three (3993) participants who were suspected of malaria, 2495 (62.5%) were reported to have malaria by the rapid diagnostic test (RDT). The rate was higher in Agona Swedru Municipality (54.5%) and Abura-Asebu-Kwamankese District reported the lowest rate of 20%. The sensitivity of clinical suspicion of malaria was between 58.2% as observed in Abura-Asebu-Kwamankese District to 68% in Ewim Polyclinic. The proportions of the suspected malaria participants that were found to have malaria by RDT in Saint Francis Xavier Hospital, Saint Gregory Catholic Hospital, Ewim Polyclinic, Abura-Asebu-Kwamankese District and Agona Swedru Municipality attributable to malaria were 20%, 32.7%, 37.8%, 46.1% and 54.5% respectively (Table 7).

Table 7: Percentage distribution of Malaria by RDT and Microscopy

Study facilities	Number of attendees	Number suspected of malaria n (%)	Number with malaria detected by RDT	Proportion with malaria	
				Percentage (%) of total attendees	Percentage (%) of suspected malaria
AAK	529	182	106	20.0	58.2
ASM	660	613	360	54.5	58.7
ACM	1203	676	393	32.7	58.1
GED	872	530	330	37.8	62.3
CCM	729	494	336	46.1	68.0
Total	3993	2495	1525	38.2	61.1

Data are presented as numbers and percentages.
AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality
Source: Field Work 2021

Participants with malaria in the study sites stratified by age and patient type

Assin Central Municipality reported the highest number of participants in four out of six of the age groupings. Of particular interest is its high prevalence (n = 60) in participants five years and below. An equal prevalence was recorded for the Gomoa East District. However, the Cape Coast Metropolis (n = 5) had the lowest malaria cases among children under the age of five years. Age group 10- 14 (n=29) was the least infected with malaria. Among the age group 20-59 years, 293 participants were from Agona Swedru Municipality, followed by 216 from Assin Central Municipality, with Abura-Asebu-Kwamankese District (n=57) recording the least prevalence in this category (Figure 19). The analysis revealed a statistically significant relationship between age group and study districts [Pearson chi2 (20) = 152.5506; *P*-value <0.001].

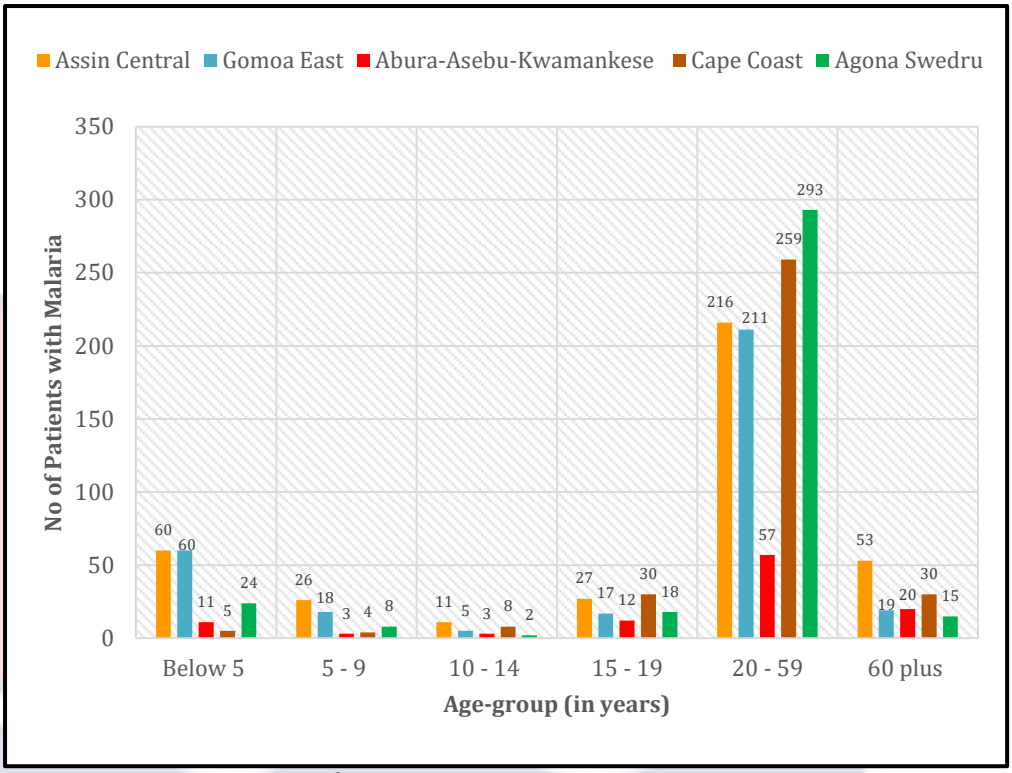


Figure 19: Distribution of participants with malaria according to age ranges and study districts.
Statistically significant relationship between age group and study districts [Pearson chi2 (20) = 152.5506; *P*-value <0.001] was observed..
Source: Field Work 2021

Gender distribution of participants with malaria in the study sites

It was also observed that the female gender dominated the malaria case categories in all the sites that were involved in the study. Further analysis shows a statistically significant association between study districts and participants with respect to gender [Pearson chi2 (4) = 47.6087; *P-value <0.001]* (Figure 20).

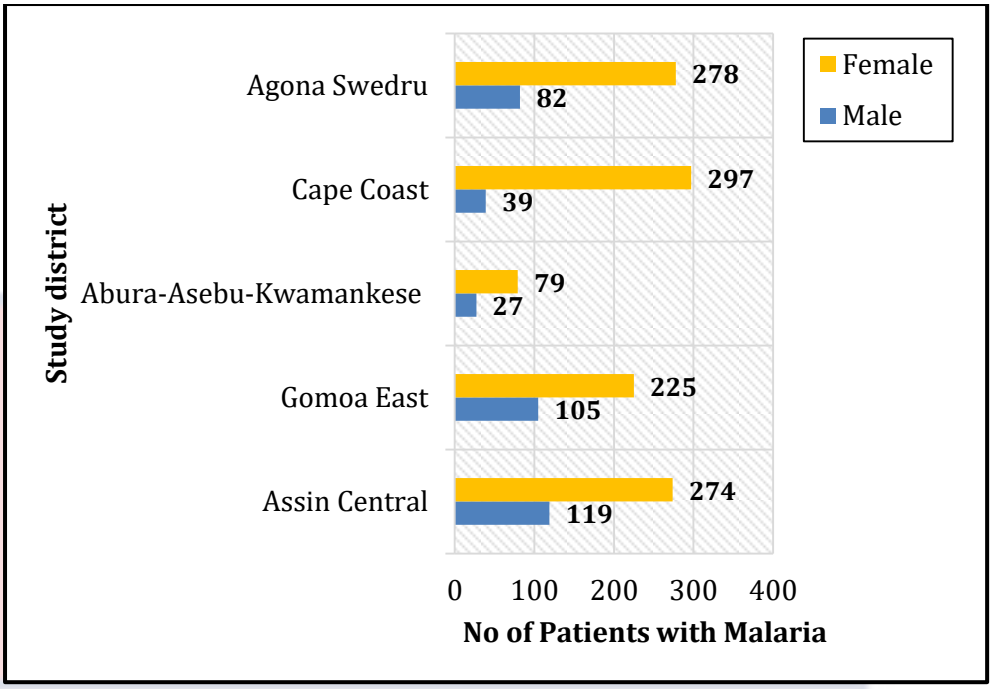


Figure 20: Distribution of participants with malaria according to gender and study districts.
Source: Field Work 2021

Distribution of participants with malaria according to patient type and study districts

Study sites where the OPD attendants with malaria dominated were Gomoa East District, whilst Abura-Asebu-Kwamankese District had the least number of OPD attendants with malaria. Among in-patients with malaria, participants in Assin Central Municipality inched past participants in Agona Swedru Municipality, while very few (n=10) in-patients with malaria were observed in Abura-Asebu-Kwamankese District. Surprisingly, of the 875 ANC attendants, 456 (52.1%) had malaria. The highest proportion was observed in Agona Swedru Municipality (62.7%) while a lower rate was observed in Abura-Asebu-Kwamankese District (37%) (Figure 21).

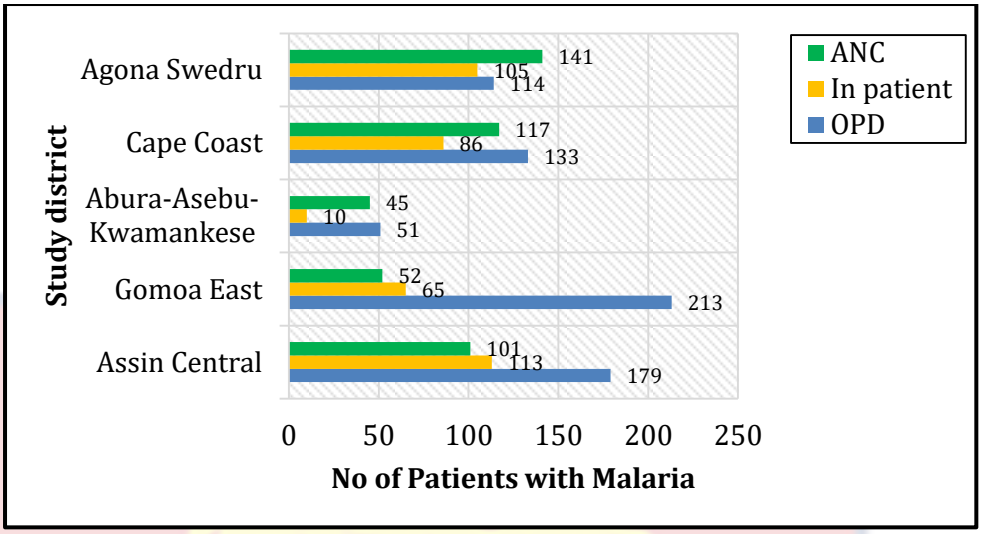


Figure 21: Distribution of participants with malaria according to patient type and study districts.
There is a statistically significant association between study districts and patient type [Pearson chi2 (8) = 106.3029; *P-value*<0.001]
Source: Field Work 2021

Comparison of Malaria incidence in ecological zones and seasons

The distribution within the forest zones (n=2758, 68.5%) account for little more than twice the number of participants recruited in the coastal zones (n=1258, 31.5%). During the dry season, 425 participants (10.6%) in the coastal zone were recruited. Of those, 191 (45%) had malaria, while the remaining participants did not. In addition, 833 participants were recruited in the rainy season in the coastal zone with 251 (30.1%) infected with malaria. Out of the 539 participants coming from the forest zones, 245 (45.5%) of them reported malaria during the dry season. Additionally, malaria affected 838 (38.2%) of the 2196 participants recruited during the rainy season in the forest zone. In both zones, the prevalence of malaria was found to be higher during the dry season than during the rainy season. Analysis also shows a statistically significant association between seasons and ecological zones recording a *P*-value < 0.001 in both coastal zone and forest zones. In a similar vein, among the negative cases, there was a statistically significant relationship between

seasons and ecological zones, with a *P*-value of 0.001 in the coastal zone and 0.005 in the forest zone (Table 8).

Table 8: Ecological and Seasonal distribution of malaria cases

Outcome of malaria testing	**Positive**			**Negative**		
	Season			**Season**		
Ecology	Dry	Rainy	*P-value*	Dry	Rainy	*P-value*
Coastal (n=1258)	191 (45%)	251 (30.1%)	< 0.001**	234 (55%)	582 (69.9%)	< 0.001**
Forest (n=2735)	245 (45.5%)	838 (38.2%)	< 0.001**	294 (54.5%)	1358 (61.8%)	0.005*

Distribution of participants with malaria according to ecology and seasons.
Data are presented as numbers and percentages
*p<0.05 and **p<0.001 was said to be statistical significant
Source: Field Work 2021

Simple logistic regression analysis of Malaria in the districts stratified by season and gender

The bivariate analysis showed that seasonal variation had an effect on malaria prevalence within the study sites; Abura-Asebu-Kwamankese District (*P-value* <0.001), Cape Coast Metropolis (*P-value* <0.001), and Agona Swedru Municipality (*P-value* <0.001) during the rainy season. A significant relationship was also observed in Gomoa East District (*P-value* = 0.012), Cape Coast Metropolis, and Agona Swedru Municipality (*P-value* <0.001). However, no significant relationship was observed in Abura-Asebu-Kwamankese District both in the rainy season (OR=0.48; *p- value* <0.001) and in the dry season (OR=1.02; *p- value* = 0.953) (OR=1.02; *P-value* = 0.953). These odds are however twice as much during the Rainy season (OR=2.08; *p-value* <0.001), and five times more during the dry season (OR=5.12; *p- value* <0.001) in Agona Swedru Municipality when compared to the reference district Assin Central Municipality (Table 9).

Both males and females showed a significant association with malaria prevalence in the Agona Swedru Municipality. However, only females in Abura-Asebu-Kwamankese District and Cape Coast Metropolis showed a significant association with malaria prevalence whilst only males were significantly associated with malaria prevalence in Gomoa East District. The odds of getting malaria among the gender are least among males (OR=0.79; *p-value* = 0.357) and females (OR=0.46; *p- value* <0.001) in Abura-Asebu-Kwamankese District. However, it was high in males (OR=2.16; *p- value* <0.001) and females (OR=2.58; *p- value* <0.001) in Agona Swedru Municipality when compared to the reference district Assin Central Municipality (Table 9).

Table 9: Simple logistic regression analysis of Malaria in the districts stratified by season and gender

	Seasons			
	Rainy		Dry	
District	COR [95% CI]	*P-value*	COR [95% CI]	*P-value*
ACM	Ref		ref	
GED	1.18[0.96-1.45]	0.107	1.96[1.16-3.32]	0.012*
AAK	0.48[0.37-0.62]	<0.001**	1.02[0.48-2.16]	0.953
CCM	1.58[1.24-2.01]	<0.001**	2.78[1.66-4.61]	<0.001**
ASM	2.08[1.67-2.59]	<0.001**	5.12[2.97-8.85]	<0.001**
	Gender			
District	Male		Female	
ACM	Ref		ref	
GED	1.74[1.24-2.44]	0.001*	1.11[0.89-1.38]	0.349
AAK	0.79[0.48-1.30]	0.357	0.46[0.35-0.61]	<0.001**
CCM	1.43[0.90-2.26]	0.133	1.81[1.47-2.24]	<0.001**
ASM	2.16[1.48-3.16]	<0.001**	2.58[2.05-3.24]	<0.001**

*p<0.05 and **p<0.001 were said to be statistical significant
COR---Crude Odds Ratio; CI---Confidence Interval. AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality
Source: Field Work 2021

Relationship between malaria and age stratified by district

Few age groupings showed significant association with the prevalence of malaria within the respective districts. A significant relationship, as well as high odds of getting malaria, were observed in children below 5 years (OR= 1.55; *P-value* = 0.018; OR=1.84; *P-value* = 0.002) and age group 5-9 years (OR= 3.12; *P-value* <0.001; OR= 2.60; *P-value* = 0.011) in Assin Central Municipality and Gomoa East District (Table 10).

Table 10: Simple logistics regression of Malaria incidence and age stratified by Assin Central Municipality and Gomoa East District

Age group (years)	Districts			
	Assin Central Municipality		Gomoa East District	
	COR [95% CI]	*P-value*	COR [95% CI]	*P-value*
20 - 59	Ref		Ref	
Below 5	1.55[1.08-2.24]	0.018*	1.84[1.25-2.74]	0.002*
5 - 9	3.12[1.69-5.75]	<0.001**	2.60[1.25-5.41]	0.011*
10 - 14	1.04[0.50-2.17]	0.908	1.34[0.42-4.28]	0.621
15 - 19	1.23[0.75-2.02]	0.412	1.06[0.57-1.97]	0.845
60 plus	0.88[0.62-1.26]	0.485	1.02[0.57-1.83]	0.950

*p<0.05 and **p<0.001 were said to be statistically significant
COR---Crude Odds Ratio; CI---Confidence Interval. AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality
Source: Field Work 2021

A significant relationship was observed in Abura-Asebu-Kwamankese District within age groups 15-19 years (OR= 2.44; *p- value* = 0.019) and 60 plus years (OR= 2.68; *p- value* = 0.002). However, age groups and malaria prevalence showed no there was no significant relationship in the Cape Coast Metropolis (Table 11).

Table 11: Simple logistics regression of Malaria incidence and age stratified by Abura-Asebu-Kwamankese District and Cape Coast Metropolis

Age group (years)	Districts			
	Abura-Asebu-Kwamankese		Cape Coast Metropolis	
	COR [95% CI]	*P-value*	COR [95% CI]	*P-value*
20 - 59	Ref		Ref	
Below 5	1.10[0.94-2.24]	0.798	1.98[0.47-8.35]	0.355
5 - 9	1.39[0.37-5.14]	0.624	0.68[0.20-2.34]	0.538
10 - 14	1.91[0.49-7.42]	0.351	1.19[0.44-3.20]	0.738
15 - 19	2.44[1.16-5.15]	0.019*	1.32[0.76-2.27]	0.323
60 plus	2.68[1.45-4.94]	0.002*	0.87[0.53-1.43]	0.576

*p<0.05 was said to be statistically significant
COR - Crude Odds Ratio; CI - Confidence Interval
Source: Field Work 2021

Similarly, no significant relationship between malaria and age groupings was observed in the Agona Swedru Municipality. Lower odds (OR<1) of getting malaria were common among all age groups in the Agona Swedru Municipality except the age group 15-19 years which recorded odds of 1.83 (though insignificantly association) almost two times that of the reference age group 20-59 (Table 12).

Table 12: Simple logistics regression of Malaria incidence and age stratified by Agona Swedru Municipality

Age group (years)	Districts	
	Agona Swedru Municipality	
	COR [95% CI]	*P-value*
20 - 59	Ref	
Below 5	0.93[0.50-1.71]	0.811
5 - 9	0.59[0.23-1.49]	0.266
10 - 14	0.54[0.09-3.27]	0.504
15 - 19	1.83[0.78-4.28]	0.165
60 plus	0.64[0.32-1.29]	0.213

COR- Crude Odds Ratio; CI- Confidence Interval
Source: Field Work 2021

Relationship between malaria and age stratified by ecological zone

Even though the odds of malaria infections were high in the forest zones (OR= 1.32; p = 0.022), and low among participants less below 5 years

than in the coastal zones (OR= 0.56; p = 0.048), no statistically significant association was observed among age group in both zones. Participants in the age bracket of 60 years plus also showed a significant association with malaria prevalence, particularly in the forest zones even with reduced risk of infection (OR= 0.71; p = 0.014) (Table 13).

Table 13: Simple logistics regression of Malaria incidence and age stratified by Ecological zone

Age group (years)	Ecological Zone			
	Forest zone		Coastal zone	
	COR [95% CI]	*P-value*	COR [95% CI]	*P-value*
20 - 59	ref		ref	
Below 5	1.32[1.04-1.69]	0.022*	0.56[0.32-0.99]	0.048*
5 - 9	1.89[1.25-2.86]	0.003*	0.73[0.30-1.78]	0.494
10 - 14	0.83[0.46-1.48]	0.523	1.30[0.60-2.83]	0.511
15 - 19	1.10[0.79-1.55]	0.574	1.53[0.99-2.34]	0.054
60 plus	0.71[0.54-0.93]	0.014*	1.20[0.82-1.75]	0.356

*p<0.05 was said to be statistically significant
COR- Crude Odds Ratio; CI- Confidence Interval
Source: Field Work 2021

Simple logistics regression of Malaria incidence and age stratified by season

Among the seasons, the odds of getting malaria were higher among all age groups in the rainy season but twice as much among the age group 5-9 years (OR= 2.08; *P-value* <0.001). However, the age group below 5 years recorded an OR of 1.45 with *P-value* = 0.003. In the dry season however, participants 60 years plus were at reduced risk of malaria (OR= 0.32; *P-value* <0.001). Age group 15-19 however was at high risk of malaria (OR= 1.06; *p-value* = 0.836), though the association was not significant (Table 14).

Table 14: Simple logistics regression of Malaria incidence and Age Stratified by Season

Age group (years)	Seasons			
	Rainy		Dry	
	COR [95% CI]	*P-value*	COR [95% CI]	*P-value*
20 - 59	ref		ref	
Below 5	1.45[1.13-1.84]	0.003*	0.67[0.41-1.11]	0.123
5 - 9	2.08[1.39-3.12]	<0.001**	0.68[0.28-1.65]	0.390
10 - 14	1.11[0.64-1.91]	0.706	0.68[0.28-1.65]	0.390
15 - 19	1.34[0.99-1.82]	0.057	1.06[0.61-1.83]	0.836
60 plus	1.07[0.84-1.35]	0.588	0.32[0.17-0.62]	0.001*

*p<0.05; **p<0.001 were said to be statistically significant
COR - Crude Odds Ratio; CI- Confidence Interval; ref – Reference group
Source: Field Work 2021

Relationship between malaria and age stratified by gender

High odds of getting malaria were observed among both genders in all age groupings except males and females 60 years or older. Males below 5years, 10-14 years, 15-19years, and 60years plus were not significantly associated with malaria infection. Males aged 5-9 years were significantly associated with malaria infection [OR=1.94, 95% CI=1.15>3.26]. Females of all age groups plus were not significantly associated with malaria infection (Table 15).

Table 15: Simple logistics regression of Malaria incidence and age stratified by gender

Age group (years)	Gender			
	Male		Female	
	COR [95% CI]	*P-value*	COR [95% CI]	*P-value*
20 - 59	ref		ref	
Below 5	1.38[0.99-1.92]	0.06	1.05[0.75-1.45]	0.785
5 - 9	1.94[1.15-3.26]	0.012*	1.36[0.79-2.35]	0.270
10 - 14	0.78[0.37-1.63]	0.509	1.18[0.64-2.18]	0.588
15 - 19	1.30[0.69-2.42]	0.416	1.23[0.91-1.64]	0.173
60 plus	0.99[0.68-1.45]	0.977	0.78[0.58-1.03]	0.081

*p<0.05 was said to be statistically significant
COR- Crude Odds Ratio; CI- Confidence Interval; ref – Reference group
Source: Field Work 2021

Malaria-associated risk factors among participants in the Central Region of Ghana

The unadjusted logistic regression showed that age, type of patient, season, ecological zone, and study district had a statistically significant effect on

malaria prevalence. Nevertheless, only gender showed no association with malaria. After adjusting for gender, patient type, season, ecological zone, and study district, the multiple logistic regression revealed that age has a statistically significant effect on malaria prevalence. Patients in age-group below 5 years [OR=1.149, 95% CI=1.36-2.20], 5 – 9 years [OR=2.14, 95% CI=1.46-3.14], and 15 - 19 [OR=1.48, 95% CI=1.12-1.95] years have a higher risk of 1.73, 2.14, and 1.48 compared to the reference group 20-59 respectively and were significantly associated with malaria prevalence

Also, after adjusting for age, gender, season, environment, and study district, the analysis also revealed that the type of patient had a statistically significant effect on malaria prevalence (Table 16). Antenatal participants have a high odds ratio [OR=2.16, 95% CI= 1.80-2.60] and were significantly associated with malaria. After adjusting for all other variables, ecological zones had an equally significant effect on malaria prevalence (Table 16). After controlling for all other factors, the probability of malaria prevalence was 80% higher in the coastal zone than in the forest zone. After controlling for all other variables, the study district also had a statistically significant effect on malaria prevalence (Table 16). The odds of malaria is higher in Gomoa East District [OR1.26, 95% CI=, 1.04 - 1.52] and Agona Swedru Municipality [OR=0.28, 95% CI=0.21- 0.36] compared to Assin Central Municipality whilst it is 72% less in Abura-Asebu-Kwamankese District [OR=2.36, 95% CI=1.93-2.90] as compared to Assin Central Municipality (Table 16). Notwithstanding, gender and season had no significant effect on the prevalence of malaria after adjusting for other covariates in the model.

Table 16: Unadjusted and Adjusted Logistic Regression of risk factors of Malaria prevalence among participants in the Central Region of Ghana

	Unadjusted		Adjusted	
Characteristics	OR [95% CI]	*P-value*	OR [95% CI]	*P-value*
Age group (years)				
20 - 59	ref		ref	
Below 5	1.19[0.96-1.48]	0.149	1.73[1.36-2.20]	<0.001**
5 - 9	1.61[1.12-2.32]	0.011*	2.14[1.46-3.14]	<0.001**
10 - 14	0.96[0.61-1.53]	0.879	1.34[0.83-2.16]	0.236
15 - 19	1.24[0.95-1.61]	0.118	1.48[1.12-1.95]	0.006*
60 plus	0.84[0.68-1.05]	0.130	1.19[0.94-1.51]	0.145
Gender				
Female	ref		ref	
Male	1.05[0.90-1.22]	0.539	1.17[0.99-1.39]	0.071
Patient type				
OPD	ref		ref	
Inpatient	0.83[0.71-0.96]	0.014*	0.87[0.75-1.03]	0.101
ANC	1.92[1.63-2.26]	<0.001**	2.16[1.80-2.60]	<0.001**

Source: Fieldwork 2021. *p<0.05 and **p<0.001 were said to be statistically significant. COR - Crude Odds Ratio; CI - Confidence Interval. AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality

Table 16: Unadjusted and Adjusted Logistic Regression of risk factors of Malaria prevalence among participants in Central Region of Ghana … continued

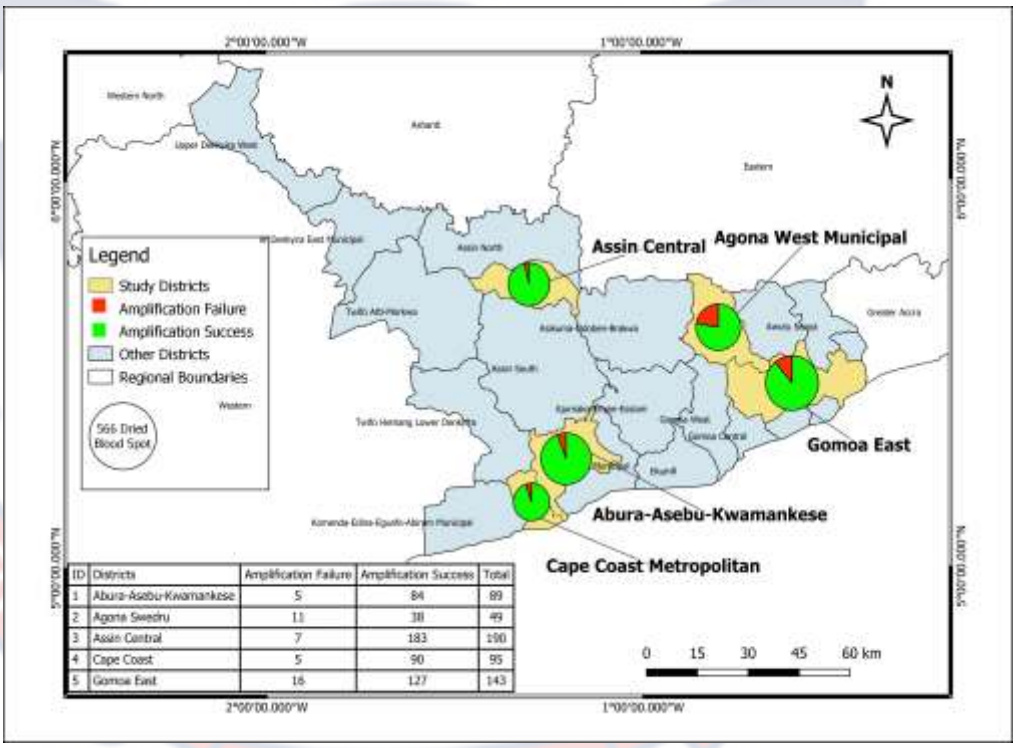
	Unadjusted		Adjusted	
Characteristics	OR [95% CI]	*P-value*	OR [95% CI]	*P-value*
Season				
Rainy	ref		ref	
Dry	1.47[1.27-1.70]	<0.001**	1.00[0.85-1.18]	0.982
Ecology				
Forest zone	ref		ref	
Coastal zone	0.83[0.72-0.95]	0.007*	1.80[1.46-2.21]	<0.001**
Study district				
ACM	ref		ref	
GED	1.25[1.05-1.51]	0.015*	1.26[1.04-1.52]	0.017*
AAK	0.52[0.40-0.66]	<0.001**	0.28[0.21-0.36]	<0.001**
CCM	1.76[1.46-2.13]	<0.001**	1	
ASM	2.47[2.04-3.01]	<0.001**	2.36[1.93-2.90]	<0.001**

Source: Fieldwork 2021. *p<0.05 and **p<0.001 were said to be statistically significant. COR - Crude Odds Ratio; CI - Confidence Interval. AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality

OBJECTIVE 2: To compare the genetic diversity of *P. falciparum* in different ecological zones and seasons

Molecular determination of the malaria parasites

A total of five hundred and sixty-six (n=566) samples collected from participants that were microscopically positive for *P. falciparum (Pf)* were analysed for parasite diversity. Whole genome amplification analysis identified *Plasmodium* infections in 92.2% (522/566) of the samples, whilst in 7.8% (44/566) of the samples, *Plasmodium* was not detected. Figure 22 shows a pie chart of gene amplification successes and failures recorded in each district.



ID	Districts	Amplification Failure	Amplification Success	Total
1	Abura-Asebu-Kwamankese	5	84	89
2	Agona Swedru	11	38	49
3	Assin Central	7	183	190
4	Cape Coast	5	90	95
5	Gomoa East	16	127	143

Figure 22: Map of study sites showing amplification success and failure.
Source: Fieldwork, 2021

***Plasmodium* species distribution in study districts**

The gene amplification revealed four species of *Plasmodium*. These were *P. falciparum* 516 (98.9%), mixed infection of *P. falciparum* and *P. ovale* 4 (0.8%), and mixed infection of *P. falciparum*, *P. vivax* and *P. malariae* 2

(0.4%). The distribution of *Plasmodium* species found in the study sites among various age groups is shown in Table 17. The only species that was found in the Agona Swedru Municipality and the Gomoa East District was *P. falciparum*, which was the species that was found to be the most common in each of the study districts. *P. falciparum* together with *P. vivax* and *P. malariae* were identified in Abura-Asebu-Kwamankese District (AAK) and the Cape Coast Metropolis. However, *P. falciparum* and *P. ovale* coinfection were identified in Assin Central Municipality. In all, more females 322 (61.7%) were infected with *P. falciparum* than males 200 (38.8%), in all the study districts (Table 17). Two (2) participants of each gender were found to have mixed infections of *P. falciparum* and *P. ovale* in Assin Central Municipality whilst a mixed infection of *P. falciparum*, *P. vivax*, and *P. malariae* was only among the males in Abura-Asebu-Kwamankese District and Cape Coast Metropolis (Table 17)

Table 17: Distribution of Species in Study District by Gender and Ecology

	AAK [c] n=84		ASM [f] n=38		CCM [c] n=90		ACM [f] n=183		GED [f] n=127		Overall n=522
	Female	Male	Female	Male	Female	Male	Female	Male	Female	Male	
	64	20	24	14	47	43	108	75	79	48	
Species	(76.2)	(23.8)	(63.2)	(36.8)	(52.2)	(47.8)	(59.0)	(41.0)	(62.2)	(37.8)	
	64	19	24	14	47	42	106	73	79	48	516
Pf	(100)	(95)	(100)	(100)	(100)	(97.7)	(98.1)	(97.3)	(100)	(100)	(98.8)
Pf/Po	0	0	0	0	0	0	2 (1.9)	2 (2.7)	0	0	4 (0.8)
Pf/Pv/Pm	0	1 (5)	0	0	0	1 (2.3)	0	0	0	0	2 (0.4)

Data are presented as numbers and proportions.
Pf - P. falciparum, Po - P. ovale, Pv - P. vivax, Pm - P. malariae
[c] Represents Coastal Ecology, [f] Represent Forest Ecology. AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality
Source: Field work, 2021

Distribution of *Plasmodium* species by ecology and gender

P. falciparum infections were more prevalent within the forest ecology (n=348, 66.7%) than in the coastal (n=174, 33.3%), and more prevalent among females than males in each ecological zone (Table 16). *P. ovale* infections were found among participants in the forest ecology, whilst *P. malariae* and *P. vivax* infections were found among participants in the coastal zone. The *Pf*/*Po* infections in the forest zone were distributed among both genders (Table 18).

Table 18: Distribution of Species by Ecology and Gender

	Ecological zone n (%)				Overall n=522
	Coastal 174 (33.3)		Forest 348 (66.7)		
Species	Female 111 (63.8)	Male 63 (36.2)	Female 211 (60.6)	Male 137 (39.4)	
Pf	111 (100)	61 (96.8)	209 (99.1)	135 (98.5)	516 (98.8)
Pf/*Po*	0	0	2 (0.95)	2 (1.5)	4 (0.8)
Pf/*Pv*/*Pm*	0	2 (3.2)	0	0	2 (0.4)

Data are presented as numbers and proportions.
Pf - P. falciparum, Po - P. ovale, Pv - P. vivax, Pm - P. malariae
Source: Fieldwork, 2021

Distribution of species by seasons and gender

More than two times the number of infections in the rainy season (152-29.1%) was recorded in the dry season 370 (70.9%). More females than males were infected in both dry (231, 62.4%) and rainy seasons (91, 59.9%). All mixed infections of *Pf*/*Pv*/*Pm* were recorded in males during the dry season. However, *Pf*/*Po* mixed infections were distributed among the gender but were more prevalent within the dry season (Table 19).

Table 19: Distribution of Species by Seasons and Gender

	Season				
	Dry n (%)		Rainy n (%)		Total n=522
Plasmodium Species	Female 231 (62.4)	Male 139 (37.6)	Female 91 (59.9)	Male 61 (40.1)	
Pf	229 (99.1)	136 (97.8)	91 (100)	60 (98.6)	516 (98.8)
Pf/*Po*	2 (0.9)	1 (0.7)	0	1 (1.6)	4 (0.8)
Pf/*Pv*/*Pm*	0	2 (1.4)	0	0	2 (0.4)

Data are presented as numbers and proportions.
Pf - P. falciparum, Po - P. ovale, Pv - P. vivax, Pm - P. malariae
Source: Fieldwork, 2021

Sequencing success and failure rates

Whole genome sequencing was carried out on 522 successfully amplified genes. Of these, 409 (78%) samples were successfully sequenced whilst 157 (22%) were not. Sequencing success ranged from 29 (7.1%) in Agona Swedru Municipality to 155 (38%) in Assin Central Municipality whilst sequencing failure ranged from 20 (12.7%) to 51 (32.5) across the study sites. Details of gene amplification successes and failures recorded in each study site are illustrated in figure 23.

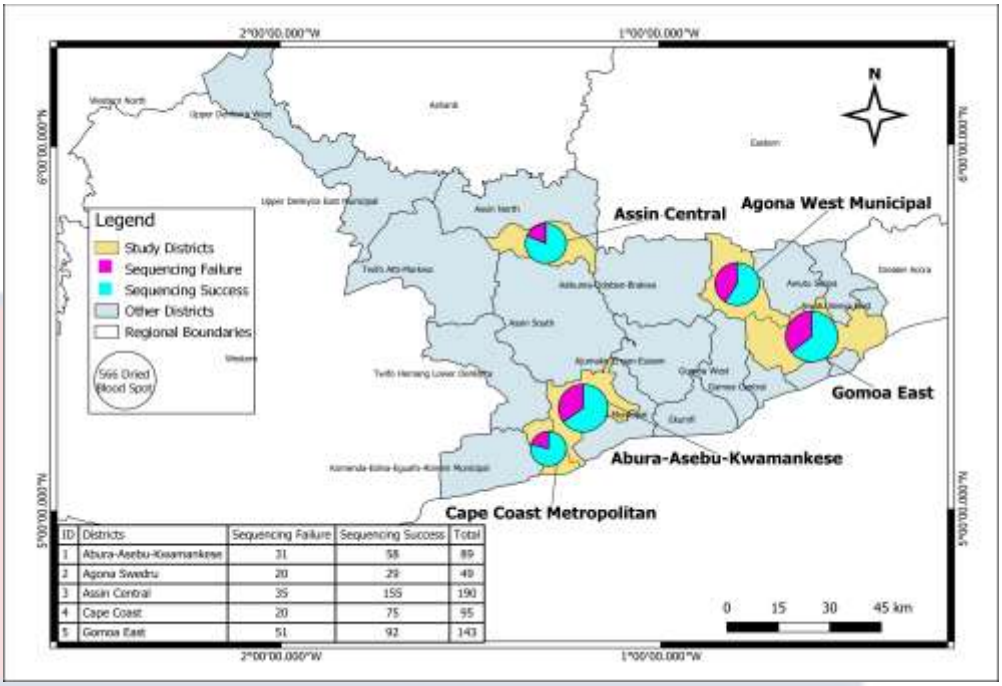


Figure 23: Map of study sites showing Sequencing success and failure. Source: Fieldwork, 2021

Distribution of clones by district

The number of parasite clones found in malaria-infected participants ranged from one to six. The frequency of monoclonal infections was 182 (44.5%) whilst polyclonal infections made up 227 (55.5%). The highest number of polyclonal infections (6 clones) were identified only in the Cape Coast Metropolis followed by five (5) clones in all other districts except in Abura-Asebu-Kwamankese District (Table 20).

Participants with monoclonal infections were the most prevalent cohort in Abura-Asebu-Kwamankese District (30), Cape Coast Metropolis (36), and Gomoa East District (44) respectively. However, in Assin Central Municipality, participants with biclonal infections (n=65, 42.0%) outnumbered those with monoclonal infections (n=60, 38.7%). An equal distribution of 12 participants (41.4%) each were observed to have either monoclonal or biclonal infections in Agona Swedru Municipality. Two participants (1.3%) were

infected with polyclonal of 5 clones in Assin Central Municipality, whilst Agona Swedru Municipality, Cape Coast Metropolis, and Gomoa East District each recorded a single patient each with a prevalence of 3.5%, 1.3%, and 1.1% respectively (Table 20). However, the distributions of the parasite clones were not statistically significant with respect to the study districts with *P-value* = 0.684 (Table 20).

Table 20: Relationship between COI and Study District

COI	Overall (N=409)	Study District					*P*-value
		AAK n (%)	ASM n (%)	CCM n (%)	ACM n (%)	GED n (%)	
1	182 (44.5)	30(51.7)	12(41.4)	36(48.0)	60(38.7)	44 (47.8)	0.684
2	163 (39.9)	24(41.4)	12(41.4)	30(40.0)	65(42.0)	32 (34.8)	
3	36(8.8)	3(5.2)	2(6.9)	3(4.0)	19(12.3)	9(9.8)	
4	22(5.4)	1(1.7)	2(6.9)	4(5.3)	9(5.8)	6(6.5)	
5	5(1.2)	0	1(3.5)	1(1.3)	2(1.3)	1(1.1)	
6	1(0.2)	0	0	1(1.3)	0	0	

Data are presented as numbers and proportions.
Statistically significant at *P-value* <0.05
AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality. COI -1 indicates monoclonal infection, COI-2 indicates biclonal infection, COI -3 to 6 indicates polyclonal infection
Source: Fieldwork, 2021

Variations of Mean COI within study districts

The mean clones by study site for *P. falciparum* isolates collected were 1.57, 1.90, 1.76, 1.89, and 1.78 for Abura-Asebu-Kwamankese District, Agona Swedru Municipality, Cape Coast Metropolis, Assin Central Municipality, and Gomoa East District respectively. Analysis of variance shows no variations in the overall mean COI among study districts (*F*-Value = 1.4; *P*=0.2344). However, Bartlett's test for equal variances shows that there are variations in COI in the various Districts (chi2 (4) = 11.2287; *P*-value = 0.024) (Table 21).

Table 21: Variations of Mean COI within study districts

Study Districts	Mean	Standard Deviation	95% Confidence Interval		F-value; *P-value*
			Lower limit	Upper limit	
AAK	1.57	0.68	1.39	1.74	
ASM	**1.90**	1.05	1.51	2.28	
CCM	1.76	1.01	1.53	1.99	1.4; 0.2344
ACM	**1.89**	0.92	1.74	2.04	
GED	1.78	0.95	1.59	1.98	

Statistically significant at *P*-value<0.05.
Bartlett's test for equal variances: chi2 (4) = 11.2287; *P-value* = 0.024
AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality
Source: Fieldwork, 2021

Overall mean Complexity of Infection (COI) by age group in all study sites

The mean COI ranged from 1.44 to 2.07 among the age groups. The highest mean COI was within the 15-19 age group (2.07, CI: 1.79-2.36). The lowest mean COI was among the 10-14 years age group (1.44, I: 0.75-1.73). Analysis of variance shows statistically significant variations in the overall mean COI among age groups (*F*-Value = 2.41; *P*=0.0362). However, Bartlett's test for equal variances shows that there are no variations in mean COI among the age groups (chi2 (5) = 5.05; *P*-value = 0.410). Table 22 shows the relationship between the mean COI and the various age groups.

Table 22: Overall Mean COI by Age-group in all study sites

Age-group (Years)	Mean	Standard Deviation	95% Confidence Interval		F-value; *P-value*
			Lower limit	Upper limit	
Less than 5	1.79	0.99	1.60	1.98	
5-9	1.94	1.01	1.69	2.18	
10-14	1.44	0.75	1.16	1.73	2.41;
15-19	2.07	0.93	1.79	2.36	0.0362*
20-59	1.71	0.86	1.57	1.84	
60 plus	2.06	0.85	1.64	2.48	

Bartlett's test for equal variances: chi2 (5) = 5.3448; *P*-value = 0.375
**P*-value<0.05 was said to be statistically significant
Source: Fieldwork, 2021

Distribution of COI by district and gender

In Abura-Asebu-Kwamankese District, 24 females and six males had monoclonal infections whilst 20 females and four males had biclonal infections. Only one male patient recorded a polyclonal infection of four clones in the Abura-Asebu-Kwamankese District. In Agona Swedru Municipality, eight females and four males were of monoclonal infections, whilst seven females and five males were of biclonal infections. Two participants, both male and female, had a polyclonal infection of three and four clones respectively whilst only one female patient recorded a polyclonal infection of five clone parasite infections. Monoclonal infections were found in 18 participants each in both gender in Cape Coast Metropolis whilst 17 females and 13 males were of polyclonal infections in Cape Coast Metropolis. The only polyclonal infections of six clones were recorded in a female patient in Cape Coast Metropolis. In Assin Central Municipality, females dominated with 31 and 41 participants, whilst males recorded 29 and 24 participants in infections with monoclonal and biclonal infections respectively. Six males (n=6) and three females (n=3) had polyclonal infection of four clones. Only two female participants recorded a polyclonal infection of five clones. Out of the 44 monoclonal infections, 24 and 20 were of females and males, respectively, whilst 21 females and 11 males made up the sum of 32 biclonal infections recorded in the Gomoa East District. Six of the nine (66.7%) polyclonal infections recorded in Gomoa East District were from females and the rest were males (Table 23). The only participant to record a polyclonal infection of five clones in the Gomoa East District was a female. Despite these

gender variations of the COI in the study districts, no statistical difference was found (Table 23).

Table 23: Distribution of Clones by District and gender

District	Clones	Overall	Gender n (%)		*P*-value
			Female	Male	
AAK (N=58)	1	30	24 (80.0)	6 (20.0)	0.060
	2	24	20 (83.3)	4 (16.7)	
	3	3	1 (33.3)	2 (66.7)	
	4	1	0.0	1 (100.0)	
ASM (N=29)	1	12	8 (66.7)	4 (33.3)	0.259
	2	12	7 (58.3)	5 (41.7)	
	3	2	0	2 (100.0)	
	4	2	2 (100.0)	0	
	5	1	1 (100.0)	0	
CCM (N=75)	1	36	18 (50.0)	18 (50.0)	0.663
	2	30	17 (56.7)	13 (43.3)	
	3	3	2 (66.7)	1 (33.3)	
	4	4	3 (75.0)	1 (25.0)	
	5	1	0	1 (100.0)	
	6	1	100.0	0	
ACM (N=155)	1	60	31 (51.7)	29 (48.3)	0.256
	2	65	41 (63.1)	24 (36.9)	
	3	19	10 (52.6)	9 (47.4)	
	4	9	3 (33.3)	6 (66.7)	
	5	2	2 (100.0)	0	
GED (N=92)	1	44	24 (54.5)	20 (45.5)	0.546
	2	32	21 (65.6)	11 (34.4)	
	3	9	6 (66.7)	3 (33.3)	
	4	6	5 (83.3)	1 (16.7)	
	5	1	1 (100.0)	0	

Data are presented as numbers and proportions
P-value<0.05 was said to be statistically significant
AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality. COI -1 indicates monoclonal infection, COI-2 indicates biclonal infection, COI -3 to 6 indicates polyclonal infection
Source: Fieldwork, 2021

Distribution of clones by ecological zones

There were 133 and 276 clones recorded in the coastal and forest ecological zones respectively. Monoclonal infections were the most prevalent in both the coastal (66- 49.6%) and forest (116- 42%) zones studied. Among

the polyclonal infection, the forest zone (n=160, 58%) recorded higher numbers than the coastal zone (n=67, 50.4%). Females also recorded the highest numbers of both single and multiclonal infections in the two ecological settings but were statistically insignificant. Despite the fact that this zone had a significantly higher number of polyclonal infections than the coastal zones, there was no significant association (*P*-value = 0.283) between the forest ecology and the complexity of the infection. There was equally no statistical relationship observed between the COI and gender in the coastal zone. The distribution and relationship of clones among the gender in the coastal and forest zones are shown in Table 24.

Table 24: Distribution and Relationship among Clones and Gender stratified by Ecological zones

Ecological zone		Clones	Overall	Gender n (%)		*P*-value
				Female	Male	
Coastal (N=133)	Monoclonal 66(49.6%)	1	66	42 (63.6)	24 (36.4)	0.643
	Polyclonal 67(50.4%)	2	54	37 (68.5)	17 (31.5)	
		3	6	3 (50.0)	3 (50.0)	
		4	5	3 (60.0)	2 (40.0)	
		5	1	0	1 (100.0)	
		6	1	1 (100.0)	0	
Forest (N=276)	Monoclonal 116 (42%)	1	116	63 (54.3)	53 (45.7)	0.283
	Polyclonal 160 (58%)	2	109	69 (63.3)	40 (36.7)	
		3	30	16 (53.3)	14 (46.7)	
		4	17	10 (58.8)	7 (41.2)	
		5	4	4 (100.0)	0	

Data are presented as numbers and proportions. The relationship between clones and gender stratified by ecological zones was evaluated using the Chi-square test. Statistically significant at *P-value*<0.05
COI -1 indicates monoclonal infection, COI-2 indicates biclonal infection
COI -3 to 6 indicates polyclonal infection
Source: Fieldwork, 2021

Distribution of clones by ecology and seasons

The coastal zone recorded a mean clone of 1.68 whilst the forest zone recorded 1.86. No statistical significance was observed for the comparison of mean COI in coastal and forest zones (*P-value* = 0.0681). The dry season recorded a mean clone of 1.81, whilst the rainy season recorded 1.79. Similarly, no statistical significance was observed between the dry and rainy seasons (*P-value* = 0.8034). The mean clones by ecological zones and seasons for *P. falciparum* isolates collected are shown in (Table 25).

Table 25: Mean Clones by Ecological zones and seasons

	Mean	Standard Deviation	95% Confidence Interval		*P-value*
			Lower limit	Upper limit	
Ecological zone					0.0681
Coastal	1.68	0.88	1.53	1.83	
Forest	1.86	0.94	1.74	1.97	
Seasons					0.8034
Dry	1.81	0.99	1.64	1.98	
Rainy	1.79	0.90	1.68	1.90	

Data were analyzed using a Two-sample t-test
Statistically significant at *P*-value<0.05
Source: Fieldwork, 2021

Mean parasite density and complexity of infection

The mean parasite density of participants with 6 clones was 737, while the mean parasite density of participants with 4 clones was 27,695. The calculated F-Value of 0.60 at a 5% level of significance shows that there is no significant relationship between the overall clones and the parasite density with p=0.7009. However, Bartlett's test for equal variances also shows that there are variations in mean clones among the parasite density (chi^2 (5) = 24.7964; *P-value*<0.001). Table 26, shows an analysis of variance in the relationship between the clones and parasite density in the study sites.

Table 26: Mean parasite density by clones

Clones	Mean	95% Confidence Interval		F-value; *P-value*
		Lower limit	Upper limit	
1	8057	18030	29975	0.6; 0.7009
2	6872	5320	8877	
3	3011	1820	4982	
4	**10247**	**4736**	**22171**	
5	1557	3613	36972	
6	**737**	-	-	

Statistically significant at *P*-value<0.05.
COI -1 indicate monoclonal infection, COI-2 indicate biclonal infection
COI -3 to 6 indicates polyclonal infection
Source: Fieldwork, 2021

OBJECTIVE 3: Investigation of proportions of mutations in *Pfcrt, Pfdhfr, Pfdhps, Pfmdr1, Pf-exonuclease, and Pfkelch 13* genes in the study districts

Analysis of markers of resistance focused on amino acid changes in the *Pfcrt* CMNVK 72–76 CVIET; *Pfdhfr* N51I, C59R, S108N and I164L; *Pfdhps* S436A, A437G, K540E, A581S/T, and A613S/T *Pfmdr1* N86Y, Y184F, and D1246Y; The entire amplified fragment for *Pfkelch13*, including codons 389–649, was tested for polymorphisms.

Antimalarial drug resistance markers in *P. falciparum*

In the study, whole genome sequencing was employed to gain further insight into the drug-resistance mutations by sequencing PCR-confirmed *P. falciparum* infections collected from August 2020– June 2021 in selected districts of the Central Region. DNA sequencing was performed to characterize SNPs in *P. falciparum* Chloroquine resistance transporter gene (*Pfcrt*), *P. falciparum* multidrug resistance 1 transporter (*Pfmdr1*), P. *falciparum* dihydrofolate reductase (*Pfdhfr*), *P. falciparum* dihydropteroate synthase (*Pfdhps*), and the *P. falciparum Kelch13* (*Pfkelch13)* genes which have been associated with resistance to Chloroquine, Sulfadoxine/pyrimethamine, Artemisinin, and its partner drugs. Thus, SNPs

causing amino acid changes in the wild-type genes; *Pfcrt* C72-V73-M74-N75-K76, *Pfmdr1* N86-Y184-D1246, *Pfdhfr* N51-C59-S108-I164, *Pfdhps* S436-A437-K540-A581-A613 and the *Pfkelch13* (capital letters representing wild type amino acids (AA) with their positions in the gene) were considered and discussed in this study. Markers found to be associated with Artemisinin (ACT) resistance, delayed clearance of parasite after ACT therapy, or reported as a candidate marker for ACT resistance as well as novel markers were of importance to this study.

Out of 409 samples sequenced, 6 of these samples could not be included in the analysis since the *Plasmodium* species identified in these samples were of mixed infections with *P.* falciparum, the main focus of the study. Therefore, on grounds of the presence of genes common to the genus, these could not be added. Thus, 403 successfully sequenced *P. falciparum* genes were analysed. The prevalence of polymorphisms in the genes was compared among the study districts. The prevalence of genes ranged from 358 (87.5%) to 403 (100%) and was analysed for known drug resistance mutations. Table 27 shows the prevalence of sequencing success of each gene per district.

Table 27: Distribution of successfully sequenced genes by districts

District	*Pfkelch13*	*Pfdhps*	*Pfcrt*	*Pfdhfr*	*Pfexo*	*Pfmdr1*
AAK	52 (14.5)	39 (12.4)	57 (14.1)	48 (13.4)	57 (14.1)	44 (13.1)
ASM	21 (5.9)	21 (6.7)	29 (7.2)	24 (6.7)	29 (7.2)	27 (8.0)
CCM	72 (20.1)	61 (19.4)	73 (18.1)	67 (18.7)	75 (18.6)	61 (18.2)
ACM	142 (39.7)	128 (40.8)	154 (38.2)	139 (38.7)	153 (38.0)	132 (39.3)
GED	71 (19.8)	65 (20.7)	90 (22.3)	81 (22.6)	89 (22.1)	72 (21.4)
Total	358 (88.8)	314 (77.9)	403 (100)	359 (89.1)	403 (100)	336 (83.4)

Data are presented as numbers and proportions
n = number of genes, AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality
Source: Fieldwork, 2021

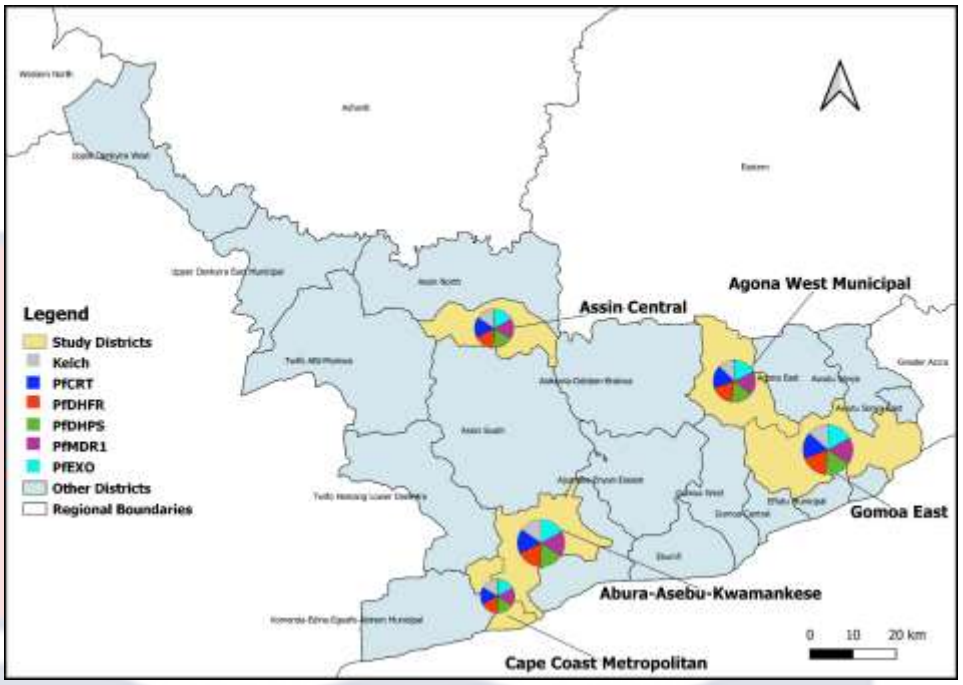


Figure 24: Map of Central Region showing gene prevalence per study site. Source: Fieldwork. 2021

Markers associated with resistance in the *Pfcrt gene*

Among the 403 successfully sequenced *Pfcrt* gene, 97% (n=391) carried the Chloroquine susceptible gene (wild type CVMNK) across the study sites, with Agona Swedru Municipality (29, 100%) recoding no case of the *Pfcrt* CVIET haplotype or mixed haplotype. However, a low prevalence in *Pfcrt* CVIET (2, 0.5%), an indicator of artemisinin susceptibility was also observed in the study. One of the two Chloroquine resistant haplotypes were found in the Cape Coast Metropolis (1, 1.4%), and the other in Assin Central Municipality (1, 0.6%). Ten 10 (2.5%) samples were recorded to have the triple mutation at codons 74(M→I), 75(N→E), and 76(K→T) as well as the wild-type haplotype. These were found in mixed haplotypes of both Chloroquine susceptible and Chloroquine-resistant markers commonly known as polyclonal haplotypes. No haplotype of SVMNT was recorded in this study (Table 28).

Table 28: Distribution of wild and mutant *Pfcrt* haplotypes by districts

		Overall	Districts n (%)				
		n=403	AAK	ASM	CCM	ACM	GED
Haplotypes			57 (14.1)	29 (7.2)	73 (18.1)	154 (38.2)	90(22.3)
Wild type	CVMNK (72C-73V-74M-75N-76K)	391 (97.0)	56 (98.2)	29 (100.0)	69 (94.5)	150 (97.4)	87 (96.7)
Mutations							
Monoclonal	CVIET (72C-73V-M75I-N75E-K76T)	2 (0.5)	0	0	1 (1.4)	1 (0.6)	0
Polyclonal	CV[M/I][N/E][K/T]	10 (2.5)	1 (1.8)	0	3 (4.1)	3 (1.9)	3 (3.3)

There is no significant association between wild and mutant *Pfcrt* gene by districts [Pearson chi^2 (8) = 4.2153; *P-value* = 0.837], n=number of haplotypes

AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality

Source: Fieldwork 2021

Distribution of *Pfcrt haplotypes* by age-groups

The Chloroquine susceptible haplotype CVMNK was most prevalent in all age groups. All children under 5 years of age (100, 100%), as well as adults 60 years (16, 100%) and above, carried the *Pfcrt* K76 CQ-sensitive marker. One isolate each in age group 5-9 years (1, 1.6%) and 20-59 years (1, 0.6), however, carried the triple mutant haplotype CVIET. The highest prevalence of mixed haplotypes was observed in participants between the age group 5–9 years (4, 6.3%), followed by participants in the age group 15-19years (2, 5.1%) and 10–14 years with 1 (3.7%). There was no polyclonal haplotype in the age group 60 years plus and children less than 5 years. The prevalence of the *Pfcrt* wild type and mutant genes had no statistically significant association (*P-value*=0.379) (Table 29).

Table 29: Prevalence of wild and mutant *Pfcrt* haplotypes stratified by patient age

			Age group (years) n (%)					
		Overall n=403	< 5	5-9	10-14	15-19	20-59	≥ 60
Haplotypes			100 (24.8)	64 (15.9)	27 (6.7)	39 (9.7)	158 (39.2)	16 (4.0)
Wild Type	CVMNK							
	(72C-73V-74M-75N-76K)	391 (97.0)	100 (100.0)	59 (92.2)	26 (96.3)	37 (94.9)	153 (96.8)	16 (100.0)
Mutations								
Monoclonal	CVIET							
	(72C-73V-M75I-N75E-K76T)	2 (0.5)	0	1 (1.6)	0	0	1 (0.6)	0.0
Polyclonal	CV[M/I][N/E][K/T]	10 (2.5)	0	4 (6.3)	1 (3.7)	2 (5.1)	3 (1.9)	0.0

There is no significant association between wild and mutant *Pfcrt* gene and age group [Pearson chi^2 (10) =10.7262; *P-value*=0.379]
n=number of haplotypes
Source: Fieldwork. 2021

Distribution of *Pfcrt haplotypes* by gender

From table 30, it was observed that the proportions of wild-type haplotypes recorded among the *Pfcrt* genes were high among males (157, 98.1%) than in females (234, 96.3%). The two CVIET haplotypes were also recorded in isolates from female (2, 0.8%) participants in this study. The proportion of mixed haplotypes in females (7, 2.9%) was higher than that in males (3, 1.9%). The proportion of haplotypes between the gender was not statistically significant (*P-value* = 0.418).

Table 30: Prevalence of wild and mutant *Pfcrt* haplotypes by gender

		Overall	Gender n (%)	
	Haplotypes	n=403	Female 243 (60.3)	Male 160 (39.7)
Wild type	CVMNK (72C-73V-74M-75N-76K)	391 (97.0)	234 (96.3)	157 (98.1)
Mutations				
Monoclonal	CVIET (72C-73V-M75I-N75E-K76T)	2 (0.5)	2 (0.8)	0
Polyclonal	CV[M/I][N/E][K/T]	10 (2.5)	7 (2.9)	3 (1.9)

Data are presented as numbers and proportions
There is no significant association between wild and mutant *Pfcrt* gene by gender [Pearson chi^2 (2) = 1.7433*; P-value* = 0.418]
n=number of mutations
Source: Fieldwork. 2021

Distribution of *Pfcrt* haplotypes by ecological zone

In all, the proportions of haplotypes in the ecological zones were higher in the forest zones (273, 67.74%) than in the coastal zone (130, 32.26%). Similarly, the prevalence of the wild-type *Pfcrt* haplotypes was high in the forest zone (266, 97.4%) than in the coastal zone (125, 96.2%). However, the Chloroquine resistant haplotype, CVIET was proportionally higher in the coastal zone (1, 0.8%) than in the forest zone (1, 0.4%). In the

case of the mixed haplotypes, higher proportions were observed in the coastal zone (4, 3.1%) compared to the forest zone (6, 2.2%). The proportions were however not statistically significant (*P-value* = 0.749) (Table 31).

Table 31: Distribution of *Pfcrt* haplotypes by Ecological zone

Haplotypes		Total N=403	Ecological zone n (%) Coastal 130 (32.26)	Forest 273 (67.74)	*P-value*
Wild type	CVMNK (72C-73V-74M-75N-76K)	391 (97.0	125 (96.2)	266 (97.4)	0.749
Mutations Monoclonal	CVIET (72C-73V-M75I-N75E-K76T)	2 (0.5)	1 (0.8)	1 (0.4)	
Polyclonal	CV[M/I][N/E][K/T]	10 (2.5)	4 (3.1)	6 (2.2)	

Data are presented as numbers and proportions
n=number of haplotypes
Source: Fieldwork. 2021

Distribution of *Pfcrt* haplotypes by seasons

Tables 32 show the distribution of the wild-type and mutant haplotypes of *Pfcrt* with respect to seasonal changes. The overall data show that haplotypes of *Pfcrt* gene were more prevalent in the rainy season (270, 67%) compared to the dry season (133, 33%). It was observed that the wild type (CVMNK) haplotype was however prevalent in the dry season (131, 98.5%) than in the rainy season (260, 96.3%). Similarly, the proportions of the CVIET haplotype were higher in the dry season (1, 0.8%) than in the rainy season (1, 0.4%). However, higher proportions of the mixed haplotype CV[M/I][N/E][K/T], were recorded in the rainy season (9, 3.3%) than in the dry season (1, 0.8%). The comparison of the proportions of the haplotypes between the dry and rainy seasons was statistically significant (Table 32).

Table 32: Distribution of *Pfcrt* haplotypes by Seasons

	Haplotypes	Total N=403	Season n (%) Dry 133 (33.00)	Rainy 270 (67.00)	*P-value*
Wild Type	CVMNK (72C-73V-74M-75N-76K)	391 (97.0)	131 (98.5)	260 (96.3)	0.259
Mutations					
Monoclonal	CVIET (72C-73V-M75I-N75E-K76T)	2 (0.5)	1 (0.8)	1 (0.4)	
Polyclonal	CV[M/I][N/E][K/T]	10 (2.5)	1 (0.8)	9 (3.3)	

Data are presented as numbers and proportions
n=number of haplotypes
Source: Fieldwork. 2021

Markers associated with resistance to *the Pfdhfr* gene

A total of 359 (89.1%) *Pfdhfr* genes of *P.falciprarum* parasites were analyzed for *Pfdhfr* genes haplotypes. Of this number, five (5, 1.4%) were of the wild type whilst the remaining were mutant haplotypes. All four (4) of the mutant alleles associated with the pyrimethamine resistance were observed in the study sites. Isolates carrying N51I, C59R, and S108N mutations in the *Pfdhfr* gene were found at a frequency of 76.6%. These were found in high proportions of 13.4% (38/48), 6.7% (18/24), 18.7% (55/67), 38.7% (100/139), and 22.6% (64/81) in Abura-Asebu-Kwamankese District, Agona Swedru Municipality, Cape Coast Metropolis, Assin Central Municipality, and Gomoa East District respectively. Mutation of Serine (S) to Asparagine (N) at codon 108 (S108N) was the most prevalent. It remained conserved in only 10 (2.8%) out of the 359 successfully sequenced genes. In all 15.0% (54) polyclonal infections were identified in the *Pfdhfr* gene. Two (0.6%) isolates were found to have the quadruple mutation N51I, C59R, S108N, and I164L in a polyclonal haplotype infection of IRN[I/L] in Assin Central Municipality.

Also, two haplotypes (2, 1.4%) were found to have a single mutation (NC[S/N]I) in a polyclonal infection in Assin Central Municipality. There is no significant association between wild and mutant *Pfdhfr* gene by districts [Pearson chi^2 (36) = 42.3626; *P*-value=0.216]. Table 33 shows the prevalence of wild-type and mutant *Pfdhfr* genes in the study district.

Table 33: Distribution of wild and mutant *Pfdhfr* haplotypes by districts

Haplotypes	Overall n=359	Districts n (%)				
		AAK 48 (13.4)	ASM 24 (6.7)	CCM 67 (18.7)	ACM 139 (38.7)	GED 81 (22.6)
Wild type						
NCSI						
51N-59C-108S-164I	5 (1.4)	0	0	2 (3.0)	3 (2.2)	0
Double Mutation						
NRNI						
51N-C59R-S108N-164I	21 (5.8)	3 (6.3)	2 (8.3)	6 (9.0)	3 (2.2)	7 (8.6)
ICNI						
N51I-59C-S108N-164I	2 (0.6)	0	1 (4.2)	0	1 (0.7)	0
Triple mutation						
IRNI						
N51I-C59R-S108N-164I	275 (76.6)	38 (79.2)	18 (75.0)	55 (82.1)	100 (71.9)	64 (79.0)
Mixed haplotypes						
[N/I]RNI	36 (10.0)	3 (6.3)	3 (12.5)	4 (6.0)	18 (12.9)	8 (9.9)
I[C/R]NI	7 (1.9)	2 (4.2)	0	0	5 (3.6)	0
IRN[I/L]	2 (0.6)	0	0	0	2 (1.4)	0
NC[S/N]I	2 (0.6)	0	0	0	2 (1.4)	0
[N/I][C/R]NI	4 (1.1)	2 (4.2)	0	0	2 (1.4)	0
[N/I][C/R][S/N]I	5 (1.4)	0	0	0	3 (2.2)	2 (2.5)

There is no significant association between wild and mutant *Pfdhfr* gene by districts [Pearson chi^2 (36) = 42.3626; *P-value* =0.216], n=number of haplotypes.

AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality

Source: Fieldwork. 2021

Distribution of *Pfdhfr* haplotypes by age-groups

Age groups less than 5 years (1, 1.1%), 15-19 years (1, 2.6%), and 20-59 (1, 0.7%) years recorded a single case each of *Pfdhfr* wild type, NCSI, whilst participants within the age group 5-9 years recorded two cases (2, 3.6%). Ages 10-14 years and ≥ 60 years recorded no wild-type haplotypes. The triple mutant IRNI was recorded in very high frequencies (greater than 50%) in all age groups but significantly high in the age groups 10-14 (20, 83.8%). However, all two cases involving the quadruple mutant mixed haplotype were recorded among the age group 15-19 years (2, 5.1%) whilst that with the single mutation was recorded in the age group 20-59years (2, 1.5%). The prevalence of the mutant *Pfdhfr* gene stratified by age group was higher than the wild type [Pearson chi^2 (230) = 65.3827; *P-value*=0.025] (Table 34).

Table 34: Prevalence of wild and mutant *Pfdhfr* haplotypes by Age group

Haplotypes	Overall n=359	Age group (years) n (%)					
		Less than 5 89 (24.8)	5-9 56 (15.6)	10-14 24 (6.7)	15-19 39 (10.9)	20-59 137 (38.2)	60 plus 14 (3.9)
Wild type							
NCSI							
51N-59C-108S-164I	5 (1.4)	1 (1.1)	2 (3.6)	0	1 (2.6)	1 (0.7)	0
Double mutation							
NRNI							
51N-C59R-S108N-164I	21 (5.8)	6 (6.7)	5 (8.9)	0	3 (7.7)	7 (5.1)	0
ICNI							
N51I-59C-S108N-164I	2 (0.6)	1 (1.1)	0	1 (4.2)	0	0	0
Triple mutation							
IRNI							
N51I-C59R-S108N-164I	275 (76.6)	70 (78.7)	40 (71.4)	20 (83.3)	26 (66.7)	111 (81.0)	8 (57.1)

There is a statistically significant association between wild and mutant *Pfdhfr* gene and age group [Pearson chi^2 (230) = 65.3827; *P-value*=0.025]
n=number of haplotypes
Source: Fieldwork. 2021

Table 34: Prevalence of wild and mutant *Pfdhfr* haplotypes by Age group … continued

Haplotypes	Overall n=359	Age group (years) n (%)					
		Less than 5 89 (24.8)	5-9 56 (15.6)	10-14 24 (6.7)	15-19 39 (10.9)	20-59 137 (38.2)	60 plus 14 (3.9)
Polyclonal haplotype	56 (15)	11 (12.4)	9 (16.1)	3 (12.5)	9 (23.1)	18 (13.1)	6 (42.9)
[N/I]RNI	36 (10.0)	9 (10.1)	5 (8.9)	3 (12.5)	3 (7.7)	11 (8.0)	5 (35.7)
I[C/R]NI	7 (1.9)	1 (1.1)	2 (3.6)	0	0	3 (2.2)	1 (7.1)
IRN[I/L]	2 (0.6)	0	0	0	2 (5.1)	0	0
NC[S/N]I	2 (0.6)	0	0	0	0	2 (1.5)	0
[N/I][C/R]NI	4 (1.1)	1 (1.1)	1 (1.8)	0	1 (2.6)	1 (0.7)	0
[N/I][C/R][S/N]I	5 (1.4)	0	1 (1.8)	0	3 (7.7)	1 (0.7)	0

There is a statistically significant association between wild and mutant *Pfdhfr* gene and age group [Pearson chi^2 (230) = 65.3827; *P-value* = 0.025]

n=number of haplotypes

Source: Fieldwork. 2021

Distribution of *Pfdhfr* haplotypes by gender

The NCSI wild-type haplotype of *Pfdhfr* and the double mutant NRNI were more prevalent in males (3, 2.0%) than in females (2, 0.9%). In females, 165/212 (77.8%) of the triple mutant haplotype IRNI were observed whilst 110/147(74.8%) were observed in males. The polyclonal haplotype containing the quadruple mutant haplotype IRN[I/L] was only recorded from female (2, 0.9%) participants of the study. However, the polyclonal haplotype containing the single mutation was unique to males (2, 1.4%) (Table 35).

Table 35: Prevalence of wild and mutant *Pfdhfr* haplotypes by gender

	Overall	Gender n (%)	
	n=359	Female	Male
Haplotypes		212 (59.1)	147 (40.9)
Wild type			
NCSI			
51N-59C-108S-164I	5 (1.4)	2 (0.9)	3 (2.0)
Double mutation			
NRNI			
51N-C59R-S108N-164I	21 (5.8)	10 (4.7)	11 (7.5)
ICNI			
N51I-59C-S108N-164I	2 (0.6)	1 (0.5)	1 (0.7)
Triple mutation			
IRNI			
N51I-C59R-S108N-164I	375 (76.6)	165 (77.8)	110 (74.8)
Polyclonal haplotypes			
[N/I]RNI	36 (10.0)	20 (9.4)	16 (10.9)
I[C/R]NI	7 (1.9)	5 (2.4)	2 (1.4)
IRN[I/L]	2 (0.6)	2 (0.9)	0
NC[S/N]I	2 (0.6)	0	2 (1.4)
[N/I][C/R]NI	4 (1.1)	2 (0.9)	2 (1.4)
[N/I][C/R][S/N]I	5 (1.4)	5 (2.4)	0

Data are presented as numbers and proportions
There is no significant association between wild and mutant *Pfdhfr* gene by gender [Pearson chi^2 (9) = 10.5550; *P-value* = 0.307]
n=number of haplotypes
Source: Fieldwork. 2021

Distribution of *Pfdhfr* haplotypes by ecological zone

The wild-type NCSI haplotype, recorded in the *Pfdhfr* gene was more prevalent in the coastal zone (2, 1.7%) than in the forest zone (3, 1.2%). The

triple mutant NRNI was also higher in the forest ecological zone (12, 4.9%) than in the coastal zone (9, 7.8%). Both the IRN[I/L] and NC[S/N]I polyclonal haplotypes were common to the forest zone (2, 0.8%) none of the haplotypes were associated with ecological zones (*P-value* = 0.341) (Tables 36).

Table 36: Distribution of *Pfdhfr* haplotypes by ecological zone

		Ecological zone n (%)		
Haplotypes	Total N=359	Coastal 115 (32.03)	Forest 244 (67.97)	*P-value*
Wild type				0.341
NCSI				
51N-59C-108S-164I	5 (1.4)	2 (1.7)	3 (1.2)	
Double Mutation				
ICNI				
N51I-59C-S108N-164I	2 (0.6)	0	2 (0.8)	
NRNI				
51N-C59R-S108N-164I	21 (5.8)	9 (7.8)	12 (4.9)	
Triple				
IRNI				
N51I-C59R-S108N-164I	275 (76.6)	93 (80.9)	182 (74.6)	
Polyclonal haplotypes				
IRN[I/L]	2 (0.6)	0	2 (0.8)	
NC[S/N]I	2 (0.6)	0	2 (0.8)	
[N/I]RNI	36 (10.0)	7 (6.1)	29 (11.9)	
I[C/R]NI	7 (1.9)	2 (1.7)	5 (2.0)	
[N/I][C/R]NI	4 (1.1)	2 (1.7)	2 (0.8)	
[N/I][C/R][S/N]I	5 (1.4)	0	5 (2.0)	

Data are presented as numbers and proportions
n=number of haplotypes
Source: Fieldwork. 2021

Distribution of *Pfdhfr* haplotypes by seasons

The wild type *Pfdhfr* was more prevalent in the dry season (3, 2.4%) than in the rainy (2, 0.9%) season. The triple mutant IRNI was observed in both seasons but recorded at a higher prevalence during the rainy season (190, 81.2%) The quadruple mutant haplotype IRN[I/L] was unique to the rainy season. *Pfdhfr* haplotypes are associated with seasonal variations (Table 37).

Table 37: Distribution of *Pfdhfr* haplotypes by Seasons

Haplotypes	Total N=359	Season n (%) Dry 125 (34.82)	Rainy 234 (65.18)	*P-value*
Wild				0.006*
NCSI				
51N-59C-108S-164I	5 (1.4)	3 (2.4)	2 (0.9)	
Double mutation				
ICNI				
N51I-59C-S108N-164I	2 (0.6)	2 (1.6)	0	
NRNI				
51N-C59R-S108N-164I	21 (5.8)	10 (8.0)	11 (4.7)	
Triple mutation				
IRNI	275			
N51I-C59R-S108N-164I	(76.6)	85 (68.0)	190(81.2%)	
Polyclonal Haplotype				
IRN[I/L]	2 (0.6)	0	2 (0.9)	
I[C/R]NI	7 (1.9)	6 (4.8)	1 (0.4)	
NC[S/N]I	2 (0.6)	2 (1.6)	0	
[N/I]RNI	36 (10.0)	13 (10.4)	23 (9.8)	
[N/I][C/R]NI	4 (1.1)	1 (1.1)	3 (1.3)	
[N/I][C/R][S/N]I	5 (1.4)	3 (2.4)	2 (0.9)	

Data are presented as numbers and proportions
n=number of haplotypes
Source: Fieldwork. 2021.

Markers of Resistance associated with *Pfdhps* gene

In the *Pfdhps* gene, the wild-type SAKAA haplotype was nearly non-existent with a prevalence of 2/314 (0.6%). The single mutants AAKAA and SGKAA were found in 1.9% of samples (6/314) and 32.8% of samples (104/314), respectively. The double mutants AGKAA and SGEAA were identified in 19.2% of samples (61/314) and 0.3% of samples (1/314), respectively. The double mutant 436G and 540E were recorded in one isolate in the Cape Coast Metropolis. Polyclonal haplotypes containing the double mutant 436G and 540E were also recorded predominantly in the Assin Central Municipality. Only one of these haplotypes was recorded in Abura-Asebu-Kwamankese District. The triple mutants AGKAS, FGKAS, and AGKSA

were found in a frequency of 21 (6.6%), 6 (1.9%), and 1 (0.3%) respectively. Two (2/314) quadruple-mutant AGKGS haplotype was identified making up 0.6% of samples. Mutation at codon 518 (518G) was recorded in the quadruple mutant AGKGS (n=2) and polyclonal haplotypes: AAK[A/G]A (n=2), AGK[A/G][A/S] (n=1) and [S/A]GK[A/G][A/S] (n=2). Mutant 518G was however not recorded in Agona Swedru Municipality and Gomoa East District (Table 38).

Table 38: Distribution of wild and mutant *Pfdhps* gene by districts

Haplotypes	Overall n=314	Districts n (%)				
		AAK 39 (12.4)	ASM 21 (6.7)	CCM 61 (19.4)	ACM 128 (40.8)	GED 65 (20.7)
Wild type						
SAKAA (436S-437A-540K-581A-613A)	2 (0.6)	0	0	1 (1.6)	1 (0.8)	0
Single mutation						
S**G**KAA (436S-A437G-540K-581A-613A)	104 (32.8)	13 (33.3)	6 (28.6)	22 (36.1)	37 (28.9)	26 (40.0)
AAKAA (S436A-437A-540K-581A-613A)	6 (1.9)	0	1 (4.8)	1 (1.6)	2 (1.6)	2 (3.1)
Double mutation						
AGKAA (S436A-A437G-540K-581A-613A)	61 (19.2)	10 (25.6)	6 (28.6)	12 (19.7)	25 (19.5)	8 (12.3)
S**GE**AA (436S-A437G-K540E-581A-613A)	1 (0.3)	0	0	1 (1.6)	0	0

There is no significant association between wild and mutant *Pfdhps* gene by districts [Pearson chi^2 (112) = 93.5338; *P-value*=0.897], n=number of haplotypes

AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality

Source: Fieldwork. 2021

Table 38: Distribution of wild and mutant *Pfdhps* gene by districts … continued

Haplotypes	Overall n=314	Districts n (%)				
		AAK 39 (12.4)	ASM 21 (6.7)	CCM 61 (19.4)	ACM 128 (40.8)	GED 65 (20.7)
Triple mutation						
AGKA**S** (S436A-A437G-540K-581A-S613S)	21 (6.6)	3 (7.7)	1 (4.8)	8 (13.1)	5 (3.9)	4 (6.2)
FGKA**S** (A436F-A437G-540K-581A-A613S)	6 (1.9)	1 (2.6)	0	1 (1.6)	2 (1.6)	2 (3.1)
AGK**S**A (S436A-A437G-540K-A581S-613A)	1 (0.3)	0	0	0	0	1 (1.5)
Quadruple mutation						
AGK**GS** (S436A-A437G-540K-A581G-A613S)	2 (0.6)	1 (2.6)	0	0	1 (0.8)	0

There is no significant association between wild and mutant *Pfdhps* gene by districts [Pearson chi^2 (112) = 93.5338; *P-value*=0.897], n=number of haplotypes

AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality

Source: Fieldwork. 2021

Distribution of wild and polyclonal *Pfdhps* haplotypes by districts

In all, one hundred and eleven (111/314, 35.4%) Polyclonal isolates were identified. The most prevalent polyclonal haplotype was [S/A]GKAA with 19.7%(62/314) mostly within Assin Central Municipality (20.3%, 26/128) and Cape Coast Metropolis (11/61, 18.0%), but the least in Agona Swedru Municipality (6/21, 28.6%). Another polyclonal haplotype found in all study sites was [S/A]GKA[A/S]. It was most prevalent in Assin Central Municipality (4, 3.1%) and a single representation in all other districts. Many unique polyclonal haplotypes were found in Assin Central Municipality compared to all the other districts (Table 39).

Table 39: Distribution of wild and polyclonal *Pfdhps* haplotypes by districts

Haplotypes	Overall n=314	Districts n (%)				
		AAK 39 (12.4)	ASM 21 (6.7)	CCM 61 (19.4)	ACM 128(40.8)	GED 65 (20.7)
Wild type						
SAKAA (436S-437A-540K-581A-613A)	2 (0.6)	0	0	1 (1.6)	1 (0.8)	0
Polyclonal haplotypes						
[S/A]GKAA	62 (19.6)	8 (20.5)	6 (28.6)	11 (18.0)	26 (20.3)	11 (16.9)
AGKA[A/S]	9 (2.8)	0.0	0	2 (3.3)	3 (2.3)	4 (6.2)
[S/A]GKA[A/S]	8 (2.5)	1 (2.6)	1 (4.8)	1 (1.6)	4 (3.1)	1 (1.5)
[S/A]G[K/E]AA	5 (1.6)	0	0	0	5 (3.9)	0
[S/A][G/A]KAA	5 (1.6)	0	0	0	4 (3.1)	1 (1.5)
[S/A][G/A][K/E]AA	3 (0.9)	0	0	0	3 (2.3)	0
SG[K/E]AA	2 (0.6)	0	0	0	2 (1.6)	0
[A/F][G/A]KAS	2 (0.6)	0	0	0	0	2 (3.1)
[A/F][G/A]KA[A/S]	2 (0.6)	0	0	0	1 (0.8)	1 (1.5)
[S/A]GK[A/G][A/S]	2 (0.6)	0	0	1 (1.6)	1 (0.8)	0
[S/A]AKAA	1 (0.3)	0	0	0	1 (0.8)	0

There is no significant association between wild and mutant *Pfdhps* gene by districts [Pearson chi^2 (112) = 93.5338; *P-value*=0.897]. n=number of haplotypes,

AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality

Source: Fieldwork. 2021

Table 39: Distribution of wild and polyclonal *Pfdhps* haplotypes by districts … continued

Haplotypes	Overall n=314	Districts n (%)				
		AAK 39 (12.4)	ASM 21 (6.7)	CCM 61 (19.4)	ACM 128(40.8)	GED 65 (20.7)
AAKA[A/T]	1 (0.3)	0	0	0	0	1 (1.5)
AAK[A/G]A	1 (0.3)	0	0	0	1 (0.8)	0
AG[K/E]AA	1 (0.3)	0	0	0	1 (0.8)	0
A[G/A]KAA	1 (0.3)	0	0	0	1 (0.8)	0
SGKA[A/S]	1 (0.3)	0	0	0	1 (0.8)	0
S[G/A]KAA	1 (0.3)	0	0	0	1 (0.8)	0
S[G/A][K/E]AA	1 (0.3)	1 (2.6)	0	0	0	0
AGK[A/G][A/S]	1 (0.3)	1 (2.6)	0	0	0	0
A[G/A]KA[A/S]	1 (0.3)	0	0	0	0	1 (1.5)

There is no significant association between wild and mutant *Pfdhps* gene by districts [Pearson chi^2 (112) = 93.5338; *P-value*=0.897].
n=number of haplotypes,
AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality
Source: Fieldwork. 2021

Distribution of *Pfdhps* monoclonal haplotypes stratified by age groups

Only two wild haplotypes of the *Pfdhps* were observed in the age group 15-19 years. The most prevalent mutant haplotype AGKAA was seen in all age groups with the highest prevalence of 29.1% (25/86) observed in the 20-59-year age group and the least prevalence of 22.2% (2/9) observed in study participants ≥ 60 years. The double mutant 436G-540E was recorded in only the age group 20-59 years. A participant each in age group 5 years and below as well as 60 years and above recorded a mutant at codon 518, observed in haplotype AGKGS. However, there was no significant association between wild and mutant *Pfdhps* gene by age group [Pearson chi^2 (140) = 137.7272; *P-value* = 0.539] (Table 40).

Table 40: Prevalence of wild and mutant *Pfdhps* monoclonal haplotypes by age group

Haplotypes	Overall n=314	Age group (years) n (%)					
		< 5	5-9	10-14	15-19	20-59	≥ 60
		52 (24.8)	32 (15.2)	13 (6.2)	18 (8.6)	86 (41.0)	9 (4.3)
Wild type							
SAKAA (436S-437A-540K-581A-613A)	2 (0.6)	2 (2.6)	0	0	0	0	0
Single mutation							
SGKAA (436S- A437G-540K-581A-613A)	104 (31.1)	30.3	18 (32.7)	10 (52.6)	8 (28.6)	43 (34.4)	2 (18.2)
AAKAA (A436A-437A-540K-581A-613A)	6 (1.9)	2 (2.6)	1 (1.8)	0	1 (3.6)	1 (0.8)	1 (9.1)
Double mutation							
AGKAA (S436A-A437G-540K-581A-613A)	61 (19.4)	61 (15.8)	12 (21.8)	4 (21.1)	4 (14.3)	27 (21.6)	2 (18.2)
SGEAA (436S-A437G-K540E-581A-613A)	1 (0.3)	0	0	1 (5.3)	0	0	0

There is no significant association between wild and mutant *Pfdhps* gene by age group [Pearson chi^2 (140) = 137.7272; *P-value*=0.539]. n=number of haplotypes.
Source: Fieldwork 2021.

Table 40: Prevalence of wild and mutant *Pfdhps* monoclonal haplotypes by age group … continued

Haplotypes	Overall n=314	Age group (years) n (%)					
		< 5	5-9	10-14	15-19	20-59	≥ 60
		52 (24.8)	32 (15.2)	13 (6.2)	18 (8.6)	86 (41.0)	9 (4.3)
Triple mutation							
AGKAS (S436A-A437G-540K-581A-A613S)	21 (6.7)	3 (3.9)	7 (12.7)	0	4 (14.3)	5 (4.0)	2 (18.2)
FGKAS (S436A-A437G-540K-581A-A613S)	6 (1.9)	2 (2.6)	0	0	1 (3.6)	3 (2.4)	0
AGKSA (S436A-A437G-540K-A581S-613A)	1 (0.3)	0	1 (1.8)	0	0	0	0
Quadruple mutation							
AGKGS (S436A-A437G-540K-A581G-A613S)	2 (0.6)	1 (1.3)	1 (1.8)	0	0	0	0

There is no significant association between wild and mutant *Pfdhps* gene by age group [Pearson chi^2 (140) = 137.7272; *P-value* = 0.539]. n=number of haplotypes.
Source: Fieldwork 2021.

Prevalence of wild and polyclonal mutant *Pfdhps* haplotypes by age-group

The most prevalent mutant polyclonal haplotype [S/A]GKAA was seen in all age groups with the highest prevalence of 27 (21.6%) in the age group 20-59 years and the least prevalence of 2 (10.5%) from participants 10-14 years and above. However, the prevalence of [S/A]GKAA among participants less than 5 years of age was high with 17 isolates (22.4%). No significant association was found between *Pfdhps* haplotypes and age group [Pearson chi^2 (140) = 137.7272; *P-value* = 0.539] (Table 41).

Table 41: Prevalence of wild and polyclonal mutant *Pfdhps* haplotypes by age group

Haplotypes	Overall n=314	Age group (years) n (%)					
		Less than 5 52 (24.8)	5-9 32 (15.2)	10-14 13 (6.2)	15-19 18 (8.6)	20-59 86 (41.0)	60 plus 9 (4.3)
Wild type							
SAKAA	2 (1.0)	0	0	0	2 (11.1)	0	0
Polyclonal haplotypes							
[S/A]GKAA	62 (19.7)	17 (22.4)	7 (12.7)	2 (10.5)	6 (21.4)	27 (21.6)	3 (27.3)
AGKA[A/S]	9 (2.9)	3 (3.9)	2 (3.6)	1 (5.3)	1 (3.6)	2 (1.6)	0
[S/A]GKA[A/S]	8 (2.5)	2 (2.6)	1 (1.8)	0	0	5 (4.0)	0
[S/A]G[K/E]AA	5 (1.6)	2 (2.6)	2 (3.6)	0	0	1 (0.8)	0
[S/A][G/A]KAA	5 (1.6)	2 (2.6)	0	0	0	3 (2.4)	0
[S/A][G/A][K/E]AA	3 (1.0)	1 (1.3)	1 (1.8)	0	1 (3.6)	0	0
SG[K/E]AA	2 (0.6)	1 (1.3)	0	0	1 (3.6)	0	0
[A/F][G/A]KAS	2 (0.6)	0	0	0	0	2 (1.6)	0
[A/F][G/A]KA[A/S]	2 (0.6)	0	0	1 (5.3)	0	1 (0.8)	0
[S/A]GK[A/G][A/S]	2 (0.6)	0	2 (3.6)	0	0	0	0

There is no significant association between wild and mutant *Pfdhps* gene by age group [Pearson chi^2 (140) = 137.7272; P=0.539]
n=number of haplotypes
Source: Fieldwork. 2021

Table 41: Prevalence of wild and polyclonal mutant *Pfdhps* haplotypes by age group … continued

Haplotypes	Overall n=314	Age group (years) n (%)					
		Less than 5 52 (24.8)	5-9 32 (15.2)	10-14 13 (6.2)	15-19 18 (8.6)	20-59 86 (41.0)	60 plus 9 (4.3)
[S/A]AKAA	1 (0.3)	0	0	0	0	1 (0.8)	0
AAKA[A/T]	1 (0.3)	0	0	0	0	1 (0.8)	0
AAK[A/G]A	1 (0.3)	1 (1.3)	0	0	0	0	0
AG[K/E]AA	1 (0.3)	1 (1.3)	0	0	0	0	0
A[G/A]KAA	1 (0.3)	0	0	0	1 (3.6)	0	0
SGKA[A/S]	1 (0.3)	0	0	0	0	1 (0.8)	0
S[G/A]KAA	1 (0.3)	1 (1.3)	0	0	0	0	0
A[G/A]KA[A/S]	1 (0.3)	0	0	0	0	1 (0.8)	0
S[G/A][K/E]AA	1 (0.3)	0	0	0	0	1 (0.8)	0
AGK[A/G][A/S]	1 (0.3)	0	0	0	0	0	1 (9.1)

There is no significant association between wild and mutant *Pfdhps* gene by age group [Pearson chi^2 (140) = 137.7272; *P-value* = 0.539]
n=number of haplotypes
Source: Fieldwork. 2021

Distribution of *Pfdhps* haplotypes by gender

In the *Pfdhps* gene, the wild-type haplotype SAKAA and the triple mutant AGKGS were equally distributed between the genders. The monoclonal haplotypes AGKAS and AAKAA were recorded more in males than in females whilst the haplotype SGEAA, was recorded from a female sample. However, the haplotype AGKSA was only found in males. There is no significant association between wild and mutant *Pfdhfr* and *Pfdhps* genes by gender recording *P*-value = 0.307 and *P-value* = 0.280 respectively (Table 42).

Table 42: Prevalence of wild and mutant *Pfdhps* haplotypes by gender

	Overall	Gender n (%)	
	n=314	Female	Male
Haplotypes		184 (58.6)	130 (41.4)
Wild type			
SAKAA (436S-437A-540K-581A-613A)	2 (0.6)	1 (0.5)	1 (0.8)
Single mutation			
AAKAA (S436A-437A-540K-581A-613A)	6 (1.9)	2 (1.1)	4 (3.1)
SGKAA (436S-A437G-540K-581A-613A)	104 (33.1)	61 (33.2)	43 (33.1)
Double mutation			
AGKAA (S436A-A437G-540K-581A-613A)	61 (19.4)	43 (23.4)	18 (13.8)
SGEAA (436S-A437G-540K-581A-613A)	1 (0.3)	1 (0.5)	0
Triple mutation			
AGKAS (S436A-A437G-540K-581A-A613S)	21 (6.7)	9 (4.9)	12 (9.2)
FGKAS (S436F-A437G-540K-581A-A613S)	6 (1.9)	5 (2.7)	1 (0.8)
AGKGS (S436A-A437G-540K-A581G-A613S)	2 (0.6)	1 (0.5)	1 (0.8)
AGKSA (S436A-A437G-540K-A581S-613A)	1 (0.3)	0	1 (0.8)

Data are presented as numbers and proportions
There is no significant association between wild and mutant *Pfdhps* gene by gender [Pearson chi^2 (28) = 31.8554; *P-value* = 0.280]
n=number of haplotypes
Source: Fieldwork. 2021

Prevalence of wild and mutant *Pfdhps* polyclonal haplotypes by gender

Polyclonal haplotypes were more common in females (184, 58.6%) than in males (130, 41.4%). The polyclonal haplotype SG[K/E]AA and [A/F][G/A]KAS was more common in males (1, 0.8%) than in females (1, 0.5) in both haplotypes. The [S/A]GKAA haplotype was higher in males (27, 20.8%) than in females (35, 19%). The polyclonal haplotypes [S/A]GKA[A/S] (6, 4.6%), [S/A][G/A][K/E]AA (2, 1.5%) were found more prevalent in males than in females. However, the haplotype [A/F][G/A]KA[A/S] (2, 1.5%), AAK[A/G]A (1, 0.8%), A[G/A]KAA (1, 0.8), A[G/A]KA[A/S] (1, 0.8%)and S[G/A]KAA (1, 0.8%) was common to only males. The haplotypes [S/A]GK[A/G][A/S] (2, 1.1%), AAKA[A/T] (1, 0.5%), AGK[A/G][A/S] (1, 0.3%), AG[K/E]AA (1, 0.5%), SGKA[A/S] (1, 0.5%), S[G/A][K/E]AA (1, 0.5%) and [S/A]AKAA (1, 0.5%) were found in only females participants. However, there is no significant association between wild and mutant *Pfdhps* gene by gender recording *P-value* = 0.280 (Table 43).

Table 43: Prevalence of wild and mutant *Pfdhps* polyclonal haplotypes by gender

Haplotypes	Overall n=314	Gender n (%)	
		Female 184 (58.6)	Male 130 (41.4)
Wild type			
SAKAA (436S-437A-540K-581A-613A)	2 (0.6)	1 (0.5)	1 (0.8)
Polyclonal haplotype			
[S/A]GKAA	62 (19.7)	35 (19.0)	27 (20.8)
AGKA[A/S]	9 (2.9)	5 (2.7)	4 (3.1)
[S/A]GKA[A/S]	8 (2.5)	2 (1.1)	6 (4.6)
[S/A]G[K/E]AA	5 (1.6)	4 (2.2)	1 (0.8)
[S/A][G/A]KAA	5 (1.6)	4 (2.2)	1 (0.8)
[S/A][G/A][K/E]AA	3 (1.0)	1 (0.5)	2 (1.5)
SG[K/E]AA	2 (0.6)	1 (0.5)	1 (0.8)
[A/F][G/A]KA[A/S]	2 (0.6)	0	2 (1.5)
[S/A]GK[A/G][A/S]	2 (0.6)	2 (1.1)	0
[S/A]AKAA	1 (0.3)	1 (0.5)	0
AAKA[A/T]	1 (0.3)	1 (0.5)	0
AG[K/E]AA	1 (0.3)	1 (0.5)	0
AAK[A/G]A	1 (0.3)	0	1 (0.8)
A[G/A]KAA	1 (0.3)	0	1 (0.8)
SGKA[A/S]	1 (0.3)	1 (0.5)	0
S[G/A]KAA	1 (0.3)	0	1 (0.8)
[A/F][G/A]KAS	2 (0.6)	1 (0.5)	1 (0.8)
S[G/A][K/E]AA	1 (0.3)	1 (0.5)	0
AGK[A/G][A/S]	1 (0.3)	1 (0.5)	0
A[G/A]KA[A/S]	1 (0.3)	0	1 (0.8)

Data are presented as numbers and proportions

There is no significant association between wild and mutant *Pfdhps* gene by gender [Pearson chi^2 (28) = 31.8554; *P-value* = 0.280]

n=number of haplotypes

Source: Fieldwork. 2021

Distribution of *Pfdhps* haplotypes by ecological zone

The wild-type haplotype of the *Pfdhps gene* SAKAA was found higher in the coastal zones (1, 1.0%) than in the forest zone (1, 0.5%) even though it was equally distributed in frequency between the ecological zones. The frequency of single mutant haplotype of public health importance SGKAA was almost twice as much recorded in the forest zone (69), when compared to the coastal zone (35) however the proportions in the coastal zones (35%) were

higher than in the forest zone (32.2%). The double mutation SGEAA (1, 1.0%) recorded was from a sample collected from the coastal zone. The most prevalent triple mutant haplotype AGKAS was only a single sample higher in the coastal zone (11) -than in the forest zone (10). This haplotype was also proportionally high in the coastal zone (11.0%) than in the forest zone (4.7%). Similarly, even though the quadruple mutant haplotype AGKGS was of the same frequency in the ecological zones, the haplotype was proportionally high in the coastal zone (1, 1.0%) than in the forest zones (1, 0.5%) (Table 44).

Table 44: Distribution of *Pfdhps* haplotypes by ecological zone

		Ecological zone n (%)		
	Total	Coastal	Forest	
Haplotypes	N=314	100 (31.85)	214 (68.15)	*P-value*
Wild type				0.551
SAKAA (436S-437A-540K-581A-613A)	2 (0.6)	1 (1.0)	1 (0.5)	
Single mutation				
AAKAA (S436A-437A-540K-581A-613A)	6 (1.9)	1 (1.0)	5 (2.3)	
SGKAA (436S-A437G-540K-581A-613A)	104 (33.1)	35 (35.0)	69 (32.2)	
Double mutation				
AGKAA (S436A-A437G-540K-581A-613A)	61 (19.4)	22 (22.0)	39 (18.2)	
SGEAA (436S-A437G-K540E-581A-613A)	1 (0.3)	1 (1.0)	0	
Triple mutation				
AGKAS (S436A-A437G-540K-581A-A613S)	21 (6.7)	11 (11.0)	10 (4.7)	
AGKSA (S436A-A437G-540K-A581S-613A)	1 (0.3)	0	1 (0.5)	
FGKAS (S436F-A437G-540K-581A-A613S)	6 (1.9)	2 (2.0)	4 (1.9)	
Quadruple mutation				
AGKGS (S436A-A437G-540K-A581G-A613S)	2 (0.6)	1 (1.0)	1 (0.5)	

Data are presented as numbers and proportions
n=number of haplotypes
Source: Fieldwork. 2021

Distribution of wild-type and polyclonal *Pfdhps* haplotypes by Ecological zone

The most prevalent polyclonal haplotype [S/A]GKAA was of high prevalence and proportion in the forest zones (43, 20.1%) compared to the coastal zone (19, 19.0%). However the polyclonal haplotype [S/A]GK[A/G][A/S] was proportionally high in the coastal zone (1, 1.0%) compared to the forest zone (1, 0.5%), equally distributed in both forest and coastal zones. The polyclonal haplotype AGK[A/G][A/S] (1, 1.0%) and S[G/A][K/E]AA (1, 1.0%) were unique to the coastal zone. The haplotypes [S/A]GKAA, AGKA[A/S], [S/A]GKA[A/S], [S/A]GK[A/G][A/S] were common to both the coastal and forest zones. The rest of the polyclonal haplotypes were unique to the forest zone (Table 45).

Table 45: Distribution of wild-type and polyclonal *Pfdhps* haplotypes by Ecological zone

		Ecological zone n (%)		
Haplotypes	Total N=314	Coastal 100 (31.85)	Forest 214 (68.15)	*P-value*
Wild-type				0.551
SAKAA (436S-437A-540K-581A-613A)	2 (0.6)	1 (1.0)	1 (0.5)	
Polyclonal haplotypes				
[S/A]GKAA	62 (19.7)	19 (19.0)	43 (20.1)	
AGKA[A/S]	9 (2.9)	2 (2.0)	7 (3.3)	
[S/A]GKA[A/S]	8 (2.5)	2 (2.0)	6 (2.8)	
[S/A]G[K/E]AA	5 (1.6)	0	5 (2.3)	
[S/A][G/A]KAA	5 (1.6)	0	5 (2.3)	
[S/A][G/A][K/E]AA	3 (1.0)	0	3 (1.4)	
[A/F][G/A]KAS	2 (0.6)	0	2 (0.9)	
[A/F][G/A]KA[A/S]	2 (0.6)	0	2 (0.9)	
[S/A]GK[A/G][A/S]	2 (0.6)	1 (1.0)	1 (0.5)	
AAKA[A/T]	1 (0.3)	0	1 (0.5)	
AAK[A/G]A	1 (0.3)	0	1 (0.5)	
AG[K/E]AA	1 (0.3)	0	1 (0.5)	
A[G/A]KAA	1 (0.3)	0	1 (0.5)	
SGKA[A/S]	1 (0.3)	0	1 (0.5)	
SG[K/E]AA	2 (0.6)	0	2 (0.9)	
S[G/A]KAA	1 (0.3)	0	1 (0.5)	
[S/A]AKAA	1 (0.3)	0	1 (0.5)	
AGK[A/G][A/S]	1 (0.3)	1 (1.0)	0	
A[G/A]KA[A/S]	1 (0.3)	0	1 (0.5)	
S[G/A][K/E]AA	1 (0.3)	1 (1.0)	0	

Data are presented as numbers and proportions
n=number of haplotypes
Source: Fieldwork. 2021

Distribution of *Pfdhps* monoclonal haplotypes by seasons

The wild-type haplotypes SAKAA and the quadruple mutant haplotype AGKGS were of high proportions in the dry season (1, 0.9%) than in the rainy (1, 0.5%). The double mutant SGEAA (1, 0.5%) and AGKSA (1, 0.5%) were found unique to the rainy season while high frequencies of AGKAS (15, 7.5%) and FGKAS (4, 2.0%) were recorded in the rainy season. However, the most prevalent haplotype of the *Pfdhps* SGKAA was recorded at a high frequency in the rainy season (63) than in the dry season (41). Nonetheless, the haplotype was of high proportions in the dry season (36.3%) than in the

rainy season (31.3%) haplotypes recorded in the rainy season than the dry season. The haplotype AGKAA was observed at a frequency of 39 (19.4%) and 22 (19.5%) in the rainy and dry seasons, respectively (Table 46).

Table 46: Distribution of *Pfdhps* monoclonal haplotypes by seasons

		Season n (%)		
Haplotype	Total N=314	Dry 113 (35.99)	Rainy 201 (64.01)	*P-value*
Wild Type				0.881
SAKAA (436S-437A-540K-581A-613A)	2 (0.6)	1 (0.9)	1 (0.5)	
Single				
AAKAA (S436A-437A-540K-581A-613A)	6 (1.9)	4 (3.5)	2 (1.0)	
AGKAA (S436A-A437G-540K-581A-613A)	61 (19.4)	22 (19.5)	39 (19.4)	
Double mutation				
SGEAA (436S-A437G-540K-581A-613A)	1 (0.3)	0	1 (0.5)	
SGKAA (436S-A437G-540K-581A-613A)	104(33.1)	41 (36.3)	63 (31.3)	
Triple mutation				
AGKAS (S436A-A437G-540K-581A-A613S)	21 (6.7)	6 (5.3)	15 (7.5)	
AGKSA (S436A-A437G-540K-A581S-613A)	1 (0.3)	0	1 (0.5)	
FGKAS (S436F-S437G-540K-581A-A613S)	6 (1.9)	2 (1.8)	4 (2.0)	
Quadruple mutation				
AGKGS (S436A-A437G-540K-A581G-A613S)	2 (0.6)	1 (0.9)	1 (0.5)	

Data are presented as numbers and proportions
n=number of Haplotype
Source: Fieldwork. 2021

Distribution of *Pfdhps* polyclonal haplotypes by seasons

The polyclonal haplotypes were 1.5 times more prevalent in the rainy season compared to the dry season. The dominant polyclonal haplotypes in the rainy season were, [S/A]GKAA (41, 20.4%), AGKA[A/S] (7, 3.5%), [S/A][G/A]KAA (4, 2.0%). Haplotypes AAK[A/G]A (1, 0.9%), AGK[A/G][A/S] (1, 0.9%), and [S/A]AKAA (1, 0.9%) were unique to the dry

season, whilst haplotypes [A/F][G/A]KAS (2, 1.0%), [A/F][G/A]KA[A/S] (2, 1.0%), AAKA[A/T] (1, 0.5%), AG[K/E]AA (1, 0.5%), A[G/A]KAA (1, 0.5%), SGKA[A/S] (1, 0.5%), S[G/A]KAA (1, 0.5%), A[G/A]KA[A/S] (1, 0.5%) and S[G/A][K/E]AA (1, 0.5%) were unique to the rainy season (Table 47).

Table 47: Distribution of wild type and polyclonal *Pfdhps* haplotypes by Seasons

		Season n (%)		
Haplotype	Total N=314	Dry 113 (35.99)	Rainy 201 (64.01)	*P-value*
Wild Type SAKAA (436S-437A-540K-581A-613A)	2 (0.6)	1 (0.9)	1 (0.5)	0.881
Polyclonal Haplotype				
[S/A]GKAA	62 (19.7)	21 (18.6)	41 (20.4)	
AGKA[A/S]	9 (2.9)	2 (1.8)	7 (3.5)	
[S/A]GKA[A/S]	8 (2.5)	3 (2.7)	5 (2.5)	
[S/A]G[K/E]AA	5 (1.6)	2 (1.8)	3 (1.5)	
[S/A][G/A]KAA	5 (1.6)	1 (0.9)	4 (2.0)	
[S/A][G/A][K/E]AA	3 (1.0)	2 (1.8)	1 (0.5)	
SG[K/E]AA	2 (0.6)	1 (0.9)	1 (0.5)	
[A/F][G/A]KAS	2 (0.6)	0	2 (1.0)	
[A/F][G/A]KA[A/S]	2 (0.6)	0	2 (1.0)	
[S/A]GK[A/G][A/S]	2 (0.6)	1 (0.9)	1 (0.5)	
AAKA[A/T]	1 (0.3)	0	1 (0.5)	
AAK[A/G]A	1 (0.3)	1 (0.9)	0	
AG[K/E]AA	1 (0.3)	0	1 (0.5)	
A[G/A]KAA	1 (0.3)	0	1 (0.5)	
SGKA[A/S]	1 (0.3)	0	1 (0.5)	
S[G/A]KAA	1 (0.3)	0	1 (0.5)	
[S/A]AKAA	1 (0.3)	1 (0.9)	0	
AGK[A/G][A/S]	1 (0.3)	1 (0.9)	0	
A[G/A]KA[A/S]	1 (0.3)	0	1 (0.5)	
S[G/A][K/E]AA	1 (0.3)	0	1 (0.5)	

Data are presented as numbers and proportions
n=number of Haplotype
Source: Fieldwork. 2021

Markers associated with Artemisinin resistance

Distribution of wild-type and mutant *Pfkelch13* Genes by district

Sequencing of the propeller-encoding domain of *Pfkelch13* identifies mutations that are associated with artemisinin resistance. Three hundred and fifty-eight Kelch-13 genes (358, 87.5%) were completely sequenced out of

409 isolates screened whilst the 51(12.5%) not fully sequenced. Out of these *Pfkelch13* alleles, the wild-type was found in 186 (52%) of 358 isolates. Further analyses of the sequences identified *Pfkelch13* point mutations in 172 (172/358, 48%) of isolates: 127 (127/172, 73.8%) were single mutations, whilst 42 (42/172, 24.4%) and 2 (2/172, 1.2%) were double and triple mutations, respectively. Only one (1/172, 0.6%) quadruple gene mutation was observed among the *Pfkelch13* genes. The distribution of both mutant and wild-type carriers was analysed according to the study sites (Table 48). The frequencies of the *Pfkelch13* polymorphisms in Abura-Asebu-Kwamankese District, Agona Swedru Municipality, Cape Coast Metropolis, Assin Central Municipality, and Gomoa East District were detected at 52 (14.5%), 21 (5.9%), 72 (20.1%), 142 (39.7%) and 71 (19.8%) respectively. The *Pfkelch13* polymorphisms observed were varied with some samples having more than one *Pfkelch13* allele mutation. However, all the study districts recorded a mutation in the *Pfkelch13* gene (Table 48). The *Pfkelch13* mutant A578S was found in 12 (3.4%) of 358 samples. This mutation was prevalent in all districts except Agona Swedru Municipality. In the prevailing districts, it ranged from 0.7% to 7.8%. This study also recorded at very low frequencies (0.3%) *Pfkelch13* mutant genes M579T and V568A. Mutations of A578V (n=31, 8.7 %), P413L (n=27, 7.5 %), A676D (n=18, 5.0 %), and A578S (n=12, 3.4%) were observed in high frequencies in single mutations. [Pearson chi^2 (184) = 250.0402; *P-value* = 0.001] (Table 48).

Table 48: Distribution of wild and single mutant *Pfkelch13* gene by district

	Overall	Districts n (%)				
	n=358	AAK	ASM	CCM	ACM	GED
Pfkelch13 gene		52 (14.5)	21 (5.9)	72 (20.1)	142 (39.7)	71 (19.8)
WT	186 (52.0)	19 (36.5)	10 (47.6)	41 (56.9)	93 (65.5)	23 (32.4)
Single Mutation	127 (35.4%)	23 (44.2%)	7(33.3%)	25 (61%)	40 (43%)	31 (43.7%)
A578V	**31 (8.7)**	**7 (13.5)**	**1 (4.8)**	**4 (5.6)**	**9 (6.3)**	**10 (14.1)**
P413L	**27 (7.5)**	**4 (7.7)**	**4 (19.0)**	**4 (5.6)**	**9 (6.3)**	**6 (8.5)**
A676D	**18 (5.0)**	**3 (5.8)**	**1 (4.8)**	**3 (4.2)**	**6 (4.2)**	**5 (7.0)**
A578S	**12 (3.4)**	**3 (5.8)**	**0**	**3 (4.2)**	**1 (0.7)**	**5 (7.0)**
S485G	6 (1.7)	0	0	0	6 (4.2)	0
A504D	3 (0.8)	2 (3.8)	0	0	0	1 (1.4)
A621D	3 (0.8)	0	0	0	3 (2.1)	0
L429S	3 (0.8)	1 (1.9)	0	2 (2.8)	0	0
M460V	2 (0.6)	0	0	2 (2.8)	0	0
N523S	2 (0.6)	0	0	1 (1.4)	1 (0.7)	0
S577P	2 (0.6)	0	0	1 (1.4)	1 (0.7)	0
V534A	2 (0.6)	0	0	2 (2.8)	0	0

There is a statistically significant association between wild and mutant *Pfkelch13* gene by districts [Pearson chi^2 (184) = 250.0402; *P-value* = 0.001]. n=number of *Pfkelch13 gene*. Source: Fieldwork. 2021. AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality

Table 48: Distribution of wild and single mutant *Pfkelch13* gene by district … continued

Pfkelch13 gene	Overall n=358	Districts n (%)				
		AAK 52 (14.5)	ASM 21 (5.9)	CCM 72 (20.1)	ACM 142 (39.7)	GED 71 (19.8)
E426K	1 (0.3)	0	0	0	1 (0.7)	0
E612K	1 (0.3)	1 (1.9)	0	0	0	0
F614S	1 (0.3)	0	0	0	1 (0.7)	0
G591V	1 (0.3)	0	0	0	0	1 (1.4)
I405V	1 (0.3)	0	0	1 (1.4)	0	0
I406T	1 (0.3)	1 (1.9)	0	0	0	0
I684V	1 (0.3)	0	1 (4.8)	0	0	0
K420R	1 (0.3)	1 (1.9)	0	0	0	0
K438R	1 (0.3)	0	0	0	0	1 (1.4)
R513H	1 (0.3)	0	0	1 (1.4)	0	0
V520A	1 (0.3)	0	0	0	0	1 (1.4)
V568A	**1 (0.3)**	**0**	**0**	**0**	**1 (0.7)**	**0**
M460I	1 (0.3)	0	0	0	0	1 (1.4)
M579T	1 (0.3)	0	0	0	1 (0.7)	0
V603A	1 (0.3)	0	0	0	1 (0.7)	0
W518L	1 (0.3)	0	0	1 (1.4)	0	0

There is a statistically significant association between wild and mutant *Pfkelch13* gene by districts [Pearson chi^2 (184) = 250.0402; *P-value* = 0.001]. n=number of *Pfkelch13 gene*. Source: Fieldwork. 2021. AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality

Distribution of wild and multiple *Pfkelch13* mutant genes by district

The most prevalent double mutation was A578V D516G, and most prevalent in the Gomoa East District. Only one isolate contained a validated mutation in the propeller domain associated with ACT resistance -M476I observed in South East Asia. There was a double mutation A621D M476I in an isolate from Assin Central Municipality. The mutant N537D (in double mutation A578V N537D) and V568A were also observed at an overall very low frequency. Mutations of A578V (31, 8.7 %), P413L (27, 7.5 %), A676D (18, 5.0 %), and A578S (12, 3.4%) observed in high frequencies in single mutations were also observed in combination with other variants in double, triple and quadruple mutations. Two samples recorded a triple mutation each whilst one recorded a quadruple mutation at very low frequencies (Table 49). A statistical association was observed between wild and mutant *Pfkelch13* genes by districts [Pearson chi^2 (184) = 250.0402; *P-value* = 0.001] (Table 49).

Table 49: Distribution of wild and multiple *Pfkelch13* mutations by district

	Overall	Districts n (%)				
	n=358	AAK	ASM	CCM	ACM	GED
Pfkelch13 gene		52 (14.5)	21 (5.9)	72 (20.1)	142 (39.7)	71 (19.8)
WT	186 (52.0)	19 (36.5)	10 (47.6)	41 (56.9)	93 (65.5)	23 (32.4)
Multiple mutations	45 (12.6%)	10 (19.2%)	4 (19%)	6 (8.3%)	8 (5.6%)	17 (23.9)
Double mutation						
A578V D516G	17 (4.7)	3 (5.8)	1 (4.8)	3 (4.2)	0	10 (14.1)
A578V P413L	5 (1.4)	1 (1.9)	2 (9.5)	0	1 (0.7)	1 (1.4)
A676D A578V	4 (1.1)	2 (3.8)	0	0	1 (0.7)	1 (1.4)
A676D P413L	3 (0.8)	0	0	1 (1.4)	0	2 (2.8)
A676D A504D	2 (0.6)	1 (1.9)	0	0	1 (0.7)	0
F395S K390R	2 (0.6)	0	0	1 (1.4)	1 (0.7)	0
A504D D501G	1 (0.3)	0	0	0	1 (0.7)	0
A504D P413L	1 (0.3)	1 (1.9)	0	0	0	0

There is statistically significant association between wild and mutant *Pfkelch13* gene by districts [Pearson chi^2 (184) = 250.0402; *P-value* = 0.001]

n=number of *Pfkelch13 gene*, AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality

Source: Fieldwork. 2021

Table 49: Distribution of wild and multiple *Pfkelch13* mutations by district … continued

Pfkelch13 gene	Overall n=358	Districts n (%)				
		AAK 52 (14.5)	ASM 21 (5.9)	CCM 72 (20.1)	ACM 142 (39.7)	GED 71 (19.8)
A578V N537D	**1 (0.3)**	**0**	**0**	**1 (1.4)**	**0**	**0**
A621D M476I	**1 (0.3)**	**0**	**0**	**0**	**1 (0.7)**	**0**
E651G V356A	1 (0.3)	0	0	0	0	1 (1.4)
F506L P413L	1 (0.3)	1 (1.9)	0	0	0	0
N609S I551T	1 (0.3)	0	0	0	1 (0.7)	0
W660C P413L	1 (0.3)	0	0	0	0	1 (1.4)
W660S P413L	1 (0.3)	1 (1.9)	0	0	0	0
Triple Mutations						
A578V P413L R393I	1 (0.3)	0	0	0	1 (0.7)	0
A676D A578V P413L	1 (0.3)	0	1 (4.8)	0	0	0
Quadruple Mutations						
A676D A504D D501G P413L	1 (0.3)	0	0	0	0	1 (1.4)

There is statistically significant association between wild and mutant *Pfkelch13* gene by districts [Pearson chi^2 (184) = 250.0402; *P-value* = 0.001]

n=number of *Pfkelch13 gene*, AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality

Source: Fieldwork. 2021

Distribution of single mutant *Pfkelch13* gene by age-groups

Both wild-type and single-mutant *Pfkelch13* genes were observed among all the age groups. The single mutations observed in this study were high in the age group 60plus (6, 43%) and age group 5-9 years (25, 40.3%). The least number of single mutations was recorded in the age group 15-19 years (10, 28.6%). The single mutant *Pfkelch* A578V (31, 8.7%), was found among age group 5-9years (7, 11.3%), less than 5 years (9, 9.8%), and age group 10-14years (2, 9.5%). The second most prevalent single mutation was P413L (27, 7.5%), and was common to age groups 10-14 (3, 14.3%) and 60 plus (14.3%). Other single mutant *Pfkelch13* genes were observed at low frequencies below 5% ranging from (0.3% to 2.9 %) However, there was no significant association between wild and mutant *Pfkelch13* gene among the age groups [Pearson chi^2 (230) = 214.7277; *P-value* = 0.757] (Table 50).

Table 50: Prevalence of wild and single mutant *Pfkelch13* gene by Age group

Pfkelch13 gene	Overall n=358	Age group (years) n (%)					
		Less than 5	5-9	10-14	15-19	20-59	60 plus
		92 (25.7)	62 (17.3)	21 (5.9)	35 (9.8)	134 (37.4)	14 (3.9)
Wild Type							
WT	186 (52.0)	57 (62.0)	34 (54.8)	9 (42.9)	21 (60.0)	59 (44.0)	6 (42.9)
Single mutation	127 (35.4%)	28 (30.4%)	25 (40.3%)	8 (38.1%)	10 (28.6%)	50 (37.3%)	6 (43%)
A578V	31 (8.7)	9 (9.8)	7 (11.3)	2 (9.5)	2 (5.7)	11 (8.2)	0
P413L	27 (7.5)	6 (6.5)	5 (8.1)	3 (14.3)	1 (2.9)	10 (7.5)	2 (14.3)
A676D	18 (5.0)	5 (5.4)	3 (4.8)	1 (4.8)	1 (2.9)	8 (6.0)	0
A578S	12 (3.4)	0	3 (4.8)	0	3 (8.6)	5 (3.7)	1 (7.1)
S485G	6 (1.7)	3 (3.3)	1 (1.6)	0	0.0	2 (1.5)	0
A504D	3 (0.8)	1 (1.1)	0	0	0.0	1 (0.7)	1 (7.1)
A621D	3 (0.8)	2 (2.2)	0	1 (4.8)	0.0	0	0
L429S	3 (0.8)	0	1 (1.6)	0	1 (2.9)	1 (0.7)	0
M460V	2 (0.6)	0	1 (1.6)	0	0	1 (0.7)	0
N523S	2 (0.6)	0	0	0	0	2 (1.5)	0
S577P	2 (0.6)	0	0	1 (4.8)	0	1 (0.7)	0

There is no significant association between wild and mutant *Pfkelch13* gene and age group
[Pearson chi^2 (230) = 214.7277; *P-value* = 0.757]
n=number of *Pfkelch13* gene
Source: Fieldwork. 2021

Table 50: Prevalence of wild and single mutant *Pfkelch13* gene by Age group … continued

Pfkelch13 gene	Overall n=358	Age group (years) n (%)					
		Less than 5 92 (25.7)	5-9 62 (17.3)	10-14 21 (5.9)	15-19 35 (9.8)	20-59 134 (37.4)	60 plus 14 (3.9)
V534A	2 (0.6)	0	1 (1.6)	0	0	0	1 (7.1)
E426K	1 (0.3)	0	0	0	1 (2.9)	0	0
E612K	1 (0.3)	0	1 (1.6)	0	0	0	0
F614S	1 (0.3)	0	0	0	0	1 (0.7)	0
G591V	1 (0.3)	0	0	0	0	1 (0.7)	0
I405V	1 (0.3)	0	0	0	0	1 (0.7)	0
I406T	1 (0.3)	0	0	0	0	1 (0.7)	0
I684V	1 (0.3)	0	0	0	0	1 (0.7)	0
K420R	1 (0.3)	1 (1.1)	0	0	0	0	0
K438R	1 (0.3)	0	0	0	0	0	1 (7.1)
M460I	1 (0.3)	0	0	0	0	1 (0.7)	0
M579T	1 (0.3)	1 (1.1)	0	0	0	0	0
R513H	1 (0.3)	0	0	0	1 (2.9)	0	0
V520A	1 (0.3)	0	0	0	0	1 (0.7)	0
V568A	1 (0.3)	0	0	0	0	1 (0.7)	0
V603A	1 (0.3)	0	1 (1.6)	0	0	0	0
W518L	1 (0.3)	0	1 (1.6)	0	0	0	0

There is no significant association between wild and mutant *Pfkelch13* gene and age group [Pearson chi^2 (230) = 214.7277; *P-value* = 0.757]

n=number of *Pfkelch13* gene

Source: Fieldwork. 2021

Distribution of wild and multiple *Pfkelch13* mutations by age group

There were multiple mutations observed in all age groups. The age group 10-14 years recorded the highest proportion of multiple mutations (4, 19%), closely followed by the age group 20-59 years (25, 18.7%) with the least being recorded in the age group 5-9 years (4.8%). The most prevalent double mutation was A578V D516G which was common to all age groups and most prevalent in the 60-plus age group (2, 14.3%) and the 20-59 years age group with a frequency of 11 (8.2%). A single occurrence of this *Pfkelch13* mutation was observed in age groups less than 5 years (1, 1.1%), 5-9 years (1, 1.6%), 10-14 years (1, 4.8%), and 15-19 years (1, 2.9%). The double mutation A621D M476I was found in a patient in the age group 20-59(1, 0.7%) years whilst the double mutation A578V N537D was found in the age group 5-9 years (1, 1.6%). Triple mutations were recorded in age groups less than 5 years (1, 1.1%) and 20-59 years (1, 0.7%). The only quadruple mutation was in the age group 20-59 years (1, 0.7%). There was no significant association between the mutant *Pfkelch13* gene and age group [Pearson chi^2 (230) = 214.7277; *P-value* = 0.757] (Table 51).

Table 51: Distribution of wild and multiple *Pfkelch13* mutants by Age group

	Overall	Age group (years) n (%)					
	n=358	Less than 5	5-9	10-14	15-19	20-59	60 plus
Pfkelch13 gene		92 (25.7)	62 (17.3)	21 (5.9)	35 (9.8)	134 (37.4)	14 (3.9)
Wild Type	186 (52.0)	57 (62.0)	34 (54.8)	9 (42.9)	21 (60.0)	59 (44.0)	6 (42.9)
Multiple Mutations	45 (12.6%)	7(7.6%)	3(4.8%)	4(19%)	4(11.4%)	25 (18.7%)	2 (14.3%)
Double Mutation							
A578V D516G	17 (4.7)	1 (1.1)	1 (1.6)	1 (4.8)	1 (2.9)	11 (8.2)	2 (14.3)
A578V P413L	5 (1.4)	1 (1.1)	0	2 (9.5)	1 (2.9)	1 (0.7)	0
A676D A578V	4 (1.1)	1 (1.1)	0	0	1 (2.9)	2 (1.5)	0
A676D P413L	3 (0.8)	1 (1.1)	0	0	0.0	2 (1.5)	0
A676D A504D	2 (0.6)	0	0	1 (4.8)	0	1 (0.7)	0
F395S K390R	2 (0.6)	0	1 (1.6)	0	0	1 (0.7)	0
A504D D501G	1 (0.3)	1 (1.1)	0	0	0	0	0
A504D P413L	1 (0.3)	0	0	0	0	1 (0.7)	0

There is no significant association between wild and mutant *Pfkelch13* gene and age group
[Pearson chi^2 (230) = 214.7277; *P-value* = 0.757]
n=number of *Pfkelch13* genes
Source: Fieldwork. 2021

Table 51: Distribution of wild and multiple *Pfkelch13* mutants by Age group … continued

Pfkelch13 gene	Overall n=358	Age group (years) n (%)					
		Less than 5 92 (25.7)	5-9 62 (17.3)	10-14 21 (5.9)	15-19 35 (9.8)	20-59 134 (37.4)	60 plus 14 (3.9)
A621D M476I	1 (0.3)	0	0	0	0	1 (0.7)	0
A578V N537D	1 (0.3)	0	1 (1.6)	0	0	0	0
E651G V356A	1 (0.3)	1 (1.1)	0	0	0	0	0
F506L P413L	1 (0.3)	0	0	0	0	1 (0.7)	0
N609S I551T	1 (0.3)	0	0	0	0	1 (0.7)	0
W660C P413L	1 (0.3)	0	0.0	0	0	1 (0.7)	0
W660S P413L	1 (0.3)	0	0.0	0	1 (2.9)	0	0
Triple Mutation							
A578V P413L R393I	1 (0.3)	1 (1.1)	0	0	0	0	0
A676D A578V P413L	1 (0.3)	0	0	0	0	1 (0.7)	0
Quadruple mutation							
A676D A504D D501G P413L	1 (0.3)	0	0	0	0	1 (0.7)	0

There is no significant association between wild and mutant *Pfkelch13* gene and age group [Pearson chi^2 (230) = 214.7277; *P-value* = 0.757]
n=number of *Pfkelch13* genes
Source: Fieldwork. 2021

Distribution of *Pfkelch13* gene by gender

The prevalence of the mutant and wild-type *Pfkelch13* genes were (108, 51.9%) and (100, 48.1%), respectively. From the data obtained, the wild-type *Pfkelch13* gene was higher in males, 86 (57.3%) than in females 100 (48.1%). However, there was no statistically significant difference between *Pfkelch13* mutation and gender [Pearson chi^2 (46) = 42.6863; *P-value* = 0.612]. Similarly, the proportions of the single mutant *Pfkelch13* gene were higher in males 49 (66.7%) than in females 78, (37.5%). Table 52 shows the gender distribution of wild-type and single mutants of *Pfkelch13* by gender.

Table 52: Prevalence of wild and single mutant *Pfkelch13* by gender

Pfkelch13 gene	Overall n=358	Gender n (%)	
		Female 208 (58.1)	Male 150 (41.9)
WT	186 (52.0)	100 (48.1)	86 (57.3)
Single mutant	127(68.3%)	78 (37.5%)	49 (66.7%)
A578V	31 (8.7)	20 (9.6)	11 (7.3)
P413L	27 (7.5)	17 (8.2)	10 (6.7)
A676D	18 (5.0)	10 (4.8)	8 (5.3)
A578S	12 (3.4)	9 (4.3)	3 (2.0)
S485G	6 (1.7)	3 (1.4)	3 (2.0)
A504D	3 (0.8)	2 (1.0)	1 (0.7)
A621D	3 (0.8)	1 (0.5)	2 (1.3)
L429S	3 (0.8)	1 (0.5)	2 (1.3)
M460V	2 (0.6)	0	2 (1.3)
N523S	2 (0.6)	1 (0.5)	1 (0.7)
S577P	2 (0.6)	2 (1.0)	0
V534A	2 (0.6)	1 (0.5)	1 (0.7)
E426K	1 (0.3)	0	1 (0.7)
E612K	1 (0.3)	0	1 (0.7)
F614S	1 (0.3)	1 (0.5)	0
G591V	1 (0.3)	0	1 (0.7)
I405V	1 (0.3)	1 (0.5)	0
I406T	1 (0.3)	1 (0.5)	0
I684V	1 (0.3)	1 (0.5)	0
K420R	1 (0.3)	1 (0.5)	0
K438R	1 (0.3)	1 (0.5)	0
M460I	1 (0.3)	1 (0.5)	0
M579T	1 (0.3)	0	1 (0.7)
R513H	1 (0.3)	1 (0.5)	0
V520A	1 (0.3)	1 (0.5)	0
V568A	1 (0.3)	1 (0.5)	0
V603A	1 (0.3)	1 (0.5)	0
W518L	1 (0.3)	0	1 (0.7)

There is statistically significant association between wild and mutant *Pfkelch13* gene by gender [Pearson chi^2 (46) = 42.6863; *P-value* = 0.612]
n=number of *Pfkelch13* gene
Source: Fieldwork. 2021

Distribution of wild and multiple *Pfkelch13* mutations by gender

Most of the multiple mutations of the *Pfkelch13* gene were found in females - 30 (14.4%) than males -15 (10%). Some multiple mutations were found in either gender or distributed among the gender. Mutations of public

health importance: A578V N537D (1, 0.7%) and A621D M476I (1, 0.7%) were both found in samples from male participants in this study. A sample of each gender contained a triple mutation whilst the quadruple mutation found was from a female participant (Table 53).

Table 53: Prevalence of wild and multiple mutants *Pfkelch13* gene by gender

	Overall	Gender n (%)	
	n=358	Female	Male
Pfkelch13 gene		208 (58.1)	150 (41.9)
WT	186 (52.0)	100 (48.1)	86 (57.3)
Multiple Mutations	45 (24.2%)	30 (14.4)	15 (10%)
Double mutation			
A578V D516G	17 (4.7)	9 (4.3)	8 (5.3)
A578V P413L	5 (1.4)	3 (1.4)	2 (1.3)
A676D A578V	4 (1.1)	4 (1.9)	0
A676D P413L	3 (0.8)	3 (1.4)	0
A676D A504D	2 (0.6)	1 (0.5)	1 (0.7)
F395S K390R	2 (0.6)	2 (1.0)	0
A504D D501G	1 (0.3)	0	1 (0.7)
A504D P413L	1 (0.3)	1 (0.5)	0
A578V N537D	1 (0.3)	0	1 (0.7)
A621D M476I	1 (0.3)	0	1 (0.7)
E651G V356A	1 (0.3)	1 (0.5)	0
F506L P413L	1 (0.3)	1 (0.5)	0
N609S I551T	1 (0.3)	1 (0.5)	0
W660C P413L	1 (0.3)	1 (0.5)	0
W660S P413L	1 (0.3)	1 (0.5)	0
Triple mutation			
A578V P413L R393I	1 (0.3)	0	1 (0.7)
A676D A578V P413L	1 (0.3)	1 (0.5)	0
Quadruple mutation			
A676D A504D D501G P413L	1 (0.3)	1 (0.5)	0

There is statistically significant association between wild and mutant *Pfkelch13* gene by gender [Pearson chi^2 (46) = 42.6863; *P-value* = 0. 612]
n=number of *Pfkelch13* gene
Source: Fieldwork. 2021

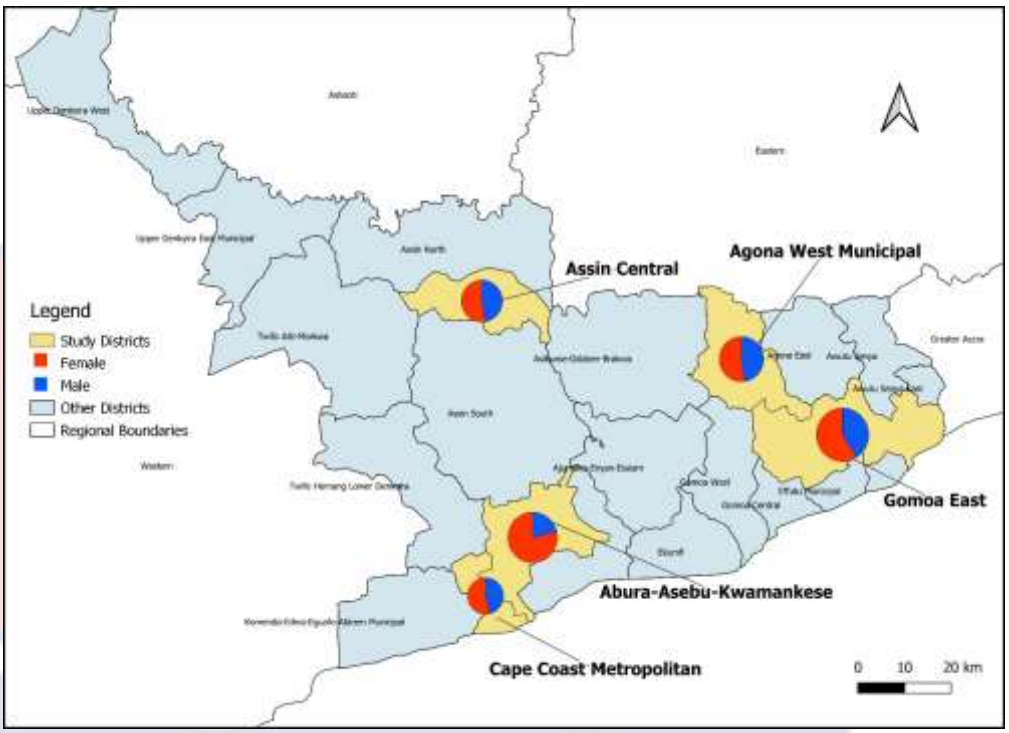


Figure 25: Prevalence of cumulative *Pfkelch13* gene by gender and district
Source: Fieldwork. 2021

Frequencies of wild and single mutant *Pfkelch13* haplotypes by the ecological zones

Wild type and mutant *Pfkelch13* distributed between the ecological zones are shown in Table 54. According to the data generated from this current study, the frequency and proportion of wild-type alleles in the forest zone (n=126, 65.36%) were found to be higher than that in the coastal zone (n=60, 48.4%). Even though the proportion of wild-type alleles was high in the forest zone compared to the coastal zone, there was no statistical significance (P- value = 0.267). The mutant gene found in high frequencies in this study: A578V, P413L, and A676D as well as double mutations containing these mutations were found in both the forest and coastal zones but of higher frequencies in the forest zone. The mutant A578S was equally distributed between the coastal and forest zones but found to be proportionally higher in

the coastal zones compared with the forest. The P413L mutant was observed at a higher proportion in the forest (19, 8.1%) than in the coast (8, 6.5%). A similar observation was made with the single mutant A676D was high in the forest (12, 5.1%) than in the coast (6, 4.8%). All six mutant S485G (6, 2.6%) were unique to the forest zone (Table 54).

Table 54: Distribution of single and mutant *Pfkelch13* gene by Ecological zone

		Ecological zone n (%)		*P-value*
Pfkelch13 gene	Total N=358	Coastal 124 (34.64)	Forest 234 (65.36)	
Wild type				0.267
WT	186 (52.0)	60 (48.4)	126 (53.8)	
Single mutation	127 (35.5%)	48(38.7%)	79 (33.8%)	
A504D	3 (0.8)	2 (1.6)	1 (0.4)	
A578S	12 (3.4)	6 (4.8)	6 (2.6)	
A578V	31 (8.7)	11 (8.9)	20 (8.5)	
A621D	3 (0.8)	0	3 (1.3)	
A676D	18 (5.0)	6 (4.8)	12 (5.1)	
E426K	1 (0.3)	0	1 (0.4)	
E612K	1 (0.3)	1 (0.8)	0	
F614S	1 (0.3)	0	1 (0.4)	
G591V	1 (0.3)	0	1 (0.4)	
I405V	1 (0.3)	1 (0.8)	0	
I406T	1 (0.3)	1 (0.8)	0	
I684V	1 (0.3)	0	1 (0.4)	
K420R	1 (0.3)	1 (0.8)	0	
K438R	1 (0.3)	0	1 (0.4)	
L429S	3 (0.8)	3 (2.4)	0	
M460I	1 (0.3)	0	1 (0.4)	
M460V	2 (0.6)	2 (1.6)	0	
M579T	1 (0.3)	0	1 (0.4)	
N523S	2 (0.6)	1 (0.8)	1 (0.4)	
P413L	27 (7.5)	8 (6.5)	19 (8.1)	
R513H	1 (0.3)	1 (0.8)	0	
S485G	6 (1.7)	0	6 (2.6)	
S577P	2 (0.6)	1 (0.8)	1 (0.4)	
V520A	1 (0.3)	0	1 (0.4)	
V534A	2 (0.6)	2 (1.6)	0	
V568A	1 (0.3)	0	1 (0.4)	
V603A	1 (0.3)	0	1 (0.4)	
W518L	1 (0.3)	1 (0.8)	0	

n=number of *Pfkelch13 genes*.
Source: Fieldwork. 2021

Distribution of wild and multiple *Pfkelch13* mutations by ecology

The prevalence of multiple mutations in the coastal zones (16, 12.9%) was slightly higher than in the forest zones (29, 12.4%). The most prevalent double mutation found, A578V D516G (17, 4.7%), was almost twice as much in the forest zone (11, 4.7%) as recorded in the coastal zone (6, 4.8%) but proportionally indifferent. Some double mutations found in very low frequencies were also found in both ecological zones. These were: A578V P413L (5, 1.4%), A676D A578V (4, 1.1%), A676D P413L (3, 0.8%), A676D A504D (2, 0.6%) and F395S K390R (2, 0.6%). All triple mutations, quadruple mutations, and double mutation A621D M476I were found to be unique to the forest zone. Some *Pfkelch13* mutant alleles were equally found to be unique to either the coastal or the forest zones but of very low frequencies (less than 5%) (Table 55).

Table 55: Distribution of wild and multiple *Pfkelch13* mutations by Ecological zones

		Ecological zone n (%)		*P-value*
	Total	Coastal	Forest	
Pfkelch13 gene	N=358	124 (34.64)	234 (65.36)	
Wild type				0.267
WT	186 (52.0)	60 (48.4)	126 (53.8)	
Double mutation	45 (12.6%)	16(12.9%)	29(12.4%)	
A504D D501G	1 (0.3)	0	1 (0.4)	
A504D P413L	1 (0.3)	1 (0.8)	0	
A578V D516G	17 (4.7)	6 (4.8)	11 (4.7)	
A578V N537D	1 (0.3)	1 (0.8)	0	
A578V P413L	5 (1.4)	1 (0.8)	4 (1.7)	
A621D M476I	1 (0.3)	0	1 (0.4)	
A676D A504D	2 (0.6)	1 (0.8)	1 (0.4)	
A676D A578V	4 (1.1)	2 (1.6)	2 (0.9)	
A676D P413L	3 (0.8)	1 (0.8)	2 (0.9)	
E651G V356A	1 (0.3)	0	1 (0.4)	
F395S K390R	2 (0.6)	1 (0.8)	1 (0.4)	
F506L P413L	1 (0.3)	1 (0.8)	0	
N609S I551T	1 (0.3)	0	1 (0.4)	
W660C P413L	1 (0.3)	0	1 (0.4)	
W660S P413L	1 (0.3)	1 (0.8)	0	
Triple mutation				
A578V P413L R393I	1 (0.3)	0	1 (0.4)	
A676D A578V P413L	1 (0.3)	0	1 (0.4)	
Quadruple mutation				
A676D A504D D501G P413L	1 (0.3)	0	1 (0.4)	

n=number of *Pfkelch13* gene. Source: Fieldwork. 2021

Distribution of *Pfkelch13* genes by seasons

Comparing the seasonal variations of the data obtained from the studied populations, the wild-type *Pfkelch13* genes were found to be higher in the dry season (66, 53.2%) than in the rainy season (120, 51.3%). The single mutant genes were found to be equally distributed in the rainy season (n=83, 35.5%) than in the dry season (n= 44, 35.5%). However, there was no significant association between these mutations and the seasons (*P-value*= 0.457) as reported in Table 56. The single mutations found in high frequencies

in this study: A578V (31, 8.7%), P413L (27, 7.5%), A676D (18, 5.0%) and A578S (12, 3.4%) were found in both the rainy and dry season but of higher frequencies in the dry season. The majority of the *Pfkelch13 genes* found in very low frequencies were also found to be unique to either the dry or the rainy season (Table 56).

Table 56: Distribution of *Pfkelch13* wild-type and single mutations by Seasons

		Season n (%)		
Mutations	Total N=358	Dry 124 (34.64)	Rainy 234 (65.36)	*P-value*
Wild-type				0.457
WT	186 (52.0)	66 (53.2)	120 (51.3)	
Single mutation	127 (35.4%)	44 (35.5%)	83 (35.5%)	
A504D	3 (0.8)	2 (1.6)	1 (0.4)	
A578S	12 (3.4)	6 (4.8)	6 (2.6)	
A578V	31 (8.7)	7 (5.6)	24 (10.3)	
A621D	3 (0.8)	1 (0.8)	2 (0.9)	
A676D	18 (5.0)	6 (4.8)	12 (5.1)	
E426K	1 (0.3)	0	1 (0.4)	
E612K	1 (0.3)	1 (0.8)	0	
F614S	1 (0.3)	0	1 (0.4)	
G591V	1 (0.3)	0	1 (0.4)	
I405V	1 (0.3)	1 (0.8)	0	
I406T	1 (0.3)	1 (0.8)	0	
I684V	1 (0.3)	1 (0.8)	0	
K420R	1 (0.3)	0	1 (0.4)	
K438R	1 (0.3)	0	1 (0.4)	
L429S	3 (0.8)	2 (1.6)	1 (0.4)	
M460I	1 (0.3)	0	1 (0.4)	
M460V	2 (0.6)	0	2 (0.9)	
M579T	1 (0.3)	0	1 (0.4)	
N523S	2 (0.6)	0	2 (0.9)	
P413L	27 (7.5)	14 (11.3)	13 (5.6)	
R513H	1 (0.3)	0	1 (0.4)	
S485G	6 (1.7)	2 (1.6)	4 (1.7)	
S577P	2 (0.6)	0	2 (0.9)	
V520A	1 (0.3)	0	1 (0.4)	
V534A	2 (0.6)	0	2 (0.9)	
V568A	1 (0.3)	0	1 (0.4)	
V603A	1 (0.3)	0	1 (0.4)	
W518L	1 (0.3)	0	1 (0.4)	

n=number of mutations
Source: Fieldwork. 2021

Distribution of wild and multiple *Pfkelch13* mutations by Seasons

The proportion of multiple mutants in the rainy season (31, 13.3%) was higher than in the dry season (14, 11.3%). The most prevalent double mutation A578V D516G was found more prevalent in the rainy season (12, 5.1%) than in the dry season (5, 4.0%). The double mutation A621D M476I (1, 0.3%), was also recorded only in the dry season. A triple mutation was recorded in each season, whilst the only recorded quadruple mutation was observed in the rainy season (Table 57).

Table 57: Distribution of *Pfkelch13* wild-type and multiple mutants by Seasons

		Season n (%)		
Pfkelch13 gene	Total N=358	Dry 124 (34.64)	Rainy 234 (65.36)	*P-value*
Wild-type				0.457
WT	186 (52.0)	66 (53.2)	120 (51.3)	
Multiple Mutation	45 (12.6%)	14(11.3%)	31 (13.3%)	
Double mutation				
A504D D501G	1 (0.3)	1 (0.8)	0	
A504D P413L	1 (0.3)	1 (0.8)	0	
A578V D516G	17 (4.7)	5 (4.0)	12 (5.1)	
A578V N537D	1 (0.3)	0	1 (0.4)	
A578V P413L	5 (1.4)	3 (2.4)	2 (0.9)	
A621D M476I	1 (0.3)	1 (0.8)	0	
A676D A504D	2 (0.6)	0	2 (0.9)	
A676D A578V	4 (1.1)	1 (0.8)	3 (1.3)	
A676D P413L	3 (0.8)	0	3 (1.3)	
E651G V356A	1 (0.3)	0	1 (0.4)	
F395S K390R	2 (0.6)	0	2 (0.9)	
F506L P413L	1 (0.3)	0	1 (0.4)	
N609S I551T	1 (0.3)	0	1 (0.4)	
W660C P413L	1 (0.3)	0	1 (0.4)	
W660S P413L	1 (0.3)	1 (0.8)	0	
Triple mutation				
A578V P413L R393I	1 (0.3)	0	1 (0.4)	
A676D A578V P413L	1 (0.3)	1 (0.8)	0	
Quadruple mutation				
A676D A504D D501G P413L	1 (0.3)	0	1 (0.4)	

n=number of *Pfkelch13* gene
Source: Fieldwork. 2021.

Markers associated with resistance to ACT partner drugs (Amodiaquine and Lumefantrine) in the *Pfmdr1* gene

Analysis of the *Pfmdr1* gene shows that the codon N86 remained as a wild-type allele in 303 (90.2%) out of the 336 successfully sequenced genes. A single isolate (1, 0.3%) contained a mixed amino acid at codon 86 (N/Y) in Abura-Asebu-Kwamankese District. At codon 184, the prevalence of wild-type Y184 was 75 (22.3%). However, Y184 was also observed in seventy-nine isolates 79 (23.5%) polyclonal infections in the *Pfmdr1* gene haplotype N[Y/F]D and an isolate (1, 0.3%) in *Pfmdr1* gene haplotype [N/Y][Y/F]D. The prevalence of *Pfmdr*1184F was 148 (44%) in haplotype (86N-Y184F-1246D), 33 (9.8%) in haplotype (N86Y-Y184F-D1246N), 79 (23.5%) in haplotype N[Y/F]D and 1 (0.3%) in haplotype [N/Y][Y/F]D. Thus, mutation at codon Y184F was the highest among the Pfmdr1 mutations.

At codon D1246, 33 (9.8) isolates mutated to D1246N in haplotype (N86Y-Y184F-D1246N). When *Pfmdr1* codons, were analysed concurrently, the wild haplotype N86-Y184-D1246, was found in 75 (22.3%) isolates. It was also found in the polyclonal haplotype N[Y/F]D (79/336, 23.5%) and [N/Y][Y/F]D (1/336, 0.3%). The prevalence of the N86Y, Y184F, and D1246Y (NFD) single mutant haplotype was 148/336 (44 %). When tallied with its prevalence in the polyclonal haplotypes N[Y/F]D (23.5%,79/336) and [N/Y][Y/F]D (1/336, 0.3%), the NFD single mutant haplotype was the most prevalent haplotype of the *Pfmdr1* gene in this study. The total prevalence of N86Y and the double mutant haplotype at amino acid positions (86 and 184) YFD was 34 (33.3%) for both haplotypes and observed in the triple mutant YFN and the polyclonal haplotype [N/Y][Y/F]D. The study site distribution of

Pfmdr1 wild and mutant haplotypes was however not found to be significantly different among the various districts (*P-value*= 0.371) (Table 58).

Table 58: Distribution of wild and mutant *Pfmdr1* haplotypes by districts

	Overall n=336	Districts n (%)				
		AAK	ASM	CCM	ACM	GED
		44	27	61	132	72
Haplotypes		(13.1)	(8.0)	(18.2)	(39.3)	(21.4)
Wild-type						
NYD	75	10	9	13	32	11
(86N-184Y-1246D)	(22.3)	(22.7)	(33.3)	(21.3)	(24.2)	(15.3)
Single mutation						
NFD	148	22	11	31	51	33
(86N-Y184F-1246D)	(44.0)	(50.0)	(40.7)	(50.8)	(38.6)	(45.8)
Triple mutation						
YFN	33		3	7	13	8
(N86Y-Y184F-D1246N)	(9.8)	2 (4.5)	(11.1)	(11.5)	(9.8)	(11.1)
Polyclonal haplotypes						
	79		4	10	36	20
N[Y/F]D	(23.5)	9 (20.5)	(14.8)	(16.4)	(27.3)	(27.8)
[N/Y][Y/F]D	1 (0.3)	1 (2.3)	0.0	0.0	0.0	0.0

There is no significant association between wild and mutant *Pfmdr1* gene by districts [Pearson chi^2 (16) = 17.2327; *P-value* = 0.371], n=number of haplotypes. AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality
Source: Fieldwork. 2021

Distribution of *Pfmdr1* genes by patient age groups

The wild-type haplotype, (86N-184Y-1246D), showed high prevalence in the age group 10-14 years (6, 27.3%), and was followed by the age group 5-9 years (23.6%). Age group 60 years plus recorded the least proportion of the wild-type haplotype (2, 15.4%). The single mutant haplotype, (86N-Y184F-1246D) was common to all age groups and most prevalent in the age group, 15-19 (16, 47.1%), closely followed by age group less than 5 years (44.6%), with the least proportion in the age group 60 years plus (5, 38.5%). The triple mutant YFN recorded a high prevalence among the age group 60 years plus (2, 15.4%) and closely followed by the age group 10-14 years (13.6%) and

children under 5 years (11, 13.3%). The least proportions of the triple mutant haplotype were found in the age group 15-19 years (1, 2.9%). The [N/Y][Y/F]D polyclonal haplotype was recorded only in the age group 20-59 years (1, 0.8%). The prevalence of the wild type and mutant haplotypes of *Pfmdr1* showed no statistical significance (*P-value*=0.995) (Table 59).

Table 59: Prevalence of wild and mutant *Pfmdr1* haplotypes by Age group

Haplotypes	Overall n=336	Age group (years) n (%)					
		Less than 5	5-9	10-14	15-19	20-59	60 plus
		83 (24.7)	55 (16.4)	22 (6.5)	34 (10.1)	129 (38.4)	13 (3.9)
Wild Type							
NYD							
(86N-184Y-1246D)	75 (22.3)	18 (21.7)	13 (23.6)	6 (27.3)	8 (23.5)	28 (21.7)	2 (15.4)
Single mutation							
NFD	148						
(86N-Y184F-1246D)	(44.0)	37 (44.6)	24 (43.6)	9 (40.9)	16 (47.1)	57 (44.2)	5 (38.5)
Triple mutation							
YFN							
(N86Y-Y184F-D1246N)	33 (9.8)	11 (13.3)	4 (7.3)	3 (13.6)	1 (2.9)	12 (9.3)	2 (15.4)
Polyclonal haplotype							
N[Y/F]D	79 (23.5)	17 (20.5)	14 (25.5)	4 (18.2)	9 (26.5)	31 (24.0)	4 (30.8)
[N/Y][Y/F]D	1 (0.3)	0	0	0	0	1 (0.8)	0

There is no significant association between wild and mutant *Pfmdr1* gene by age group [Pearson chi^2 (20) = 7.3270; *P-value*=0.995]
n=number of haplotypes
Source: Fieldwork. 2021

Distribution of *Pfmdr1* haplotypes by gender

The proportions of haplotypes were higher in females (202, 60.1%) compared to males (134, 39.9%). The wild-type haplotype was slightly higher in males (30, 22.4%) than in females (45, 22.3%). Similarly, the proportions of the haplotype NFD were high in females (92, 45.5%) compared to males (56, 41.8%). However, proportions of the triple mutant YFN and the polyclonal haplotype N[Y/F]D were higher in males [(YFN= 15 (11.2%) and N[Y/F]D = 33 (24.6%)] than in females [(YFN= 18 (8.9%) and N[Y/F]D = 46 (22.8%)]. There was no significant association between wild and mutant *Pfmdr1* gene by gender (*P-value* = 0.832). The single [N/Y][Y/F]D polyclonal haplotypes were also recorded in isolates from female (1, 0.5%) participants in this study. The distribution of *Pfmdr1* haplotypes by gender is shown in table 60.

Table 60: Prevalence of wild and mutant *Pfmdr1* haplotypes by gender

Haplotypes	Overall n=336	Gender n (%)	
		Female 202 (60.1)	Male 134 (39.9)
Wild type			
NYD (86N-184Y-1246D)	75 (22.3)	45 (22.3)	30 (22.4)
Single mutation			
NFD (86N-Y184F-1246D)	148 (44.0)	92 (45.5)	56 (41.8)
Triple mutation			
YFN (N86Y-Y184F-D1246N)	33 (9.8)	18 (8.9)	15 (11.2)
Polyclonal haplotype			
N[Y/F]D	79 (23.5)	46 (22.8)	33 (24.6)
[N/Y][Y/F]D	1 (0.3)	1 (0.5)	0

Data are presented as numbers and proportions

There is no significant association between wild and mutant *Pfmdr1* gene by gender [Pearson chi^2 (4) = 1.4669; *P*-value = 0.832]; n=number of haplotypes

Source: Fieldwork. 2021

Distribution of *Pfmdr1* haplotypes by ecological zone

In the ecological zones, the proportions of the *Pfmdr1* haplotypes were higher in the forest zone (231, 68.75%) compared to the coastal zone (105, 31.25%). The wild-type haplotype was higher in the forest (52, 2.5%) compared to the coastal (23, 21.9%). However, the single mutant haplotype was higher in the coastal zone (53, 50.5%) than in the forest zone (95, 41.1%). The triple mutant YFN, was almost three times high in frequency in the forest zone as recorded in the coastal zone and had higher proportions in the forest zone (24, 10.4%) than in the coastal zone (9, 8.6%). The polyclonal haplotype [N/Y][Y/F]D, was only found in the coastal zone, and (1, 0.3%) there was no statistical significance comparing the proportions of the coastal and forest haplotypes of the *Pfmdr1* gene (0.215) (Table 61).

Table 61: Distribution of *Pfmdr1* haplotypes by Ecological zone

		Ecological zone n (%)		
	Total	Coastal	Forest	
Haplotypes	N=336	105 (31.25)	231 (68.75)	*P-value*
Wild type NYD (86N-184Y-1246D)	75 (22.3)	23 (21.9)	**52 (22.5)**	0.215
Single mutation NFD (86N-Y184F-1246D)	148 (44.0)	**53 (50.5)**	95 (41.1)	
Triple mutation YFN (N86Y-Y184F-D1246N)	33 (9.8)	9 (8.6)	24 (10.4)	
Polyclonal Haplotype N[Y/F]D	79 (23.5)	19 (18.1)	60 (26.0)	
[N/Y][Y/F]D	1 (0.3)	1 (1.0)	0	

Data are presented as numbers and proportions
n=number of haplotypes.
Source: Fieldwork. 2021

Distribution of *Pfmdr1* by seasons

The overall prevalence of the *Pfmdr1* gene haplotypes was 112 (33.33%) in the dry season and 224 (66.67%) in the rainy season. In the *Pfmdr1* gene, the wild-type haplotype was slightly higher in the dry season (32, 28.6%) than in the rainy season (43, 19.2%). Single mutant haplotype (NFD) was of high proportions in the rainy season (106, 46.0%) compared to the dry season (45, 40.2%). Similarly, the triple mutant haplotype (YFN = 23, 10.3%) and the polyclonal haplotype N[Y/F]D (54, 24.1%) were of high prevalence during the rainy season than in the dry season (YFN = 10, 8.9% and N[Y/F]D = 25, 22.3%). The polyclonal haplotype [N/Y][Y/F]D, was also recorded in the rainy season (1, 0.4%) but not in the dry season. The distribution between the seasons showed no statistical significance (*P-value* = 0.375). Table 62 show the distribution of the wild and mutant haplotypes *Pfmdr1* with respect to the season.

Table 62: Table Distribution of *Pfmdr1* haplotypes by Seasons

		Season n (%)		
Haplotypes	Overall n=336	Dry 112 (33.33)	Rainy 224 (66.67)	*P-value*
Wild NYD (86N-184Y-1246D)	75 (22.3)	32 (28.6)	43 (19.2)	0.373
Single mutation				
NFD (86N-Y184F-1246D)	148 (44.0)	45 (40.2)	103 (46.0)	
Triple mutation				
YFN (N86Y-Y184F-D1246N)	33 (9.8)	10 (8.9)	23 (10.3)	
Polyclonal haplotypes				
N[Y/F]D	79 (23.5)	25 (22.3)	54 (24.1)	
[N/Y][Y/F]D	1 (0.4)	0	1 (0.4)	

Data are presented as numbers and proportions. n=number of haplotypes
Source: Fieldwork. 2021

Markers associated with exonuclease gene

A total of 403 parasites were successfully genotyped for *Pf-exonuclease* genes. Most samples were collected from Assin Central Municipality while the least were collected from Agona Swedru Municipality. No mutation in *Pf-exonuclease* genes was identified in this study (Table 63).

Table 63: Distribution of wild *Pf-exonuclease* genes by districts

	Overall	Districts n (%)				
	n=403	AAK	ASM	CCM	ACM	GED
Haplotype		57 (14.1)	29 (7.2)	75 (18.6)	153 (38.0)	89 (22.1)
Pf-exo	403 (100.0)	57 (100.0)	29 (100.0)	75 (100.0)	153 (100.0)	89 (100.0)

n=number of haplotypes, AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality
Source: Fieldwork. 2021

OBJECTIVE 4: Prevalence of haplotypes associated with *P. Falciparum* antimalarial drug resistance in the study districts

The molecular epidemiology investigation identified some known drug-resistant haplotypes in *P. falciparum* parasites studied. Table 64 shows Antimalarial drugs and associated markers of resistance in *P. falciparum* asexual blood-stage parasites.

Table 64: Haplotypes of mutations of epidemiological importance

Haplotype/mutation	Resistance Related drug	Found in this study	Reference(s)
CVIET	Resistant to Chloroquine	CVIET	(Asare et al., 2021)
N86Y and D1246Y	Resistance to Chloroquine and Amodiaquine Sensitive for Lumefantrine	NFD YFN	Apinjoh, et al., 2017) Mairet-Khedim et al., 2021
Pfmdr1 D1246Y existing as either NYY or haplotype YYY	Sensitive to Lumefantrine, Mefloquine, Halofantrine, and Artemisinin	NFD YFN	(Aninagyei et al., 2020) Ajogbasile, et al., 2022
Pfdhfr+Pfdhps mutations			
Partial resistance (N51I, C59R, S108N and A437G)	Resistant to Pyrimethamine Sulfadoxine	IRN-G	Svigel et al., 2021 Chaturvedi et al., 2021
Full resistance (N51I, C59R, S108N and A437G, K540E)	Resistant to Pyrimethamine Sulfadoxine	IRN-GE	Svigel et al., 2021 Chaturvedi et al., 2021
Super resistance (N51I, C59R, S108N and A437G, K540E, A581G)	Resistant to Pyrimethamine - Sulfadoxine	none	Svigel et al., 2021 Chaturvedi et al., 2021
Pfkelch 13 mutations	Resistance to artemisinin		
Validated *Pfkelch 13* mutation	F446I, N458Y, M476I, Y493 493H, R539T, I543T, P553L, R561H, P574L and C580Y	M476I	Imwong, et al., 2017
Delayed clearance		N537D V568A	(Matrevi et al., 2022)

Determinants of *Pfkelch13* resistance

Table 65 shows factors influencing *Pfkelch13* resistance in *Pfkelch13*. The unadjusted logistic regression shows that the district of stay, has a statistically significant effect on *Pfkelch13* resistance in *Pfkelch13*. Age, gender, ecology,

and season showed no statistically significant effect on *Pfkelch 13* resistance in *Pfkelch13*. The adjusted logistic regression revealed that, the risk of having *Pfkelch13* resistance alleles was 1.47 and 2.49 times more in patients aged 15 – 19 years [OR=1.47, 95% CI= 0.69 – 3.14]and 60plus [OR=2.49, CI=0.69, 8.90] years compared to age group 20-59 years. Age-group below 5 years and 10-14 years, showed a lower odds of *Pfkelch13* mutations compared to 20 - 59 years. (Table 65). The analysis also revealed that the chance of getting *Pfkelch13* resistance in *Pfkelch13* among age group 5-9years is [OR=1.03, 95% CI=0.52 - 2.03] less compared to female participants. However, the odds of getting *Pfkelch13* mutation is comparable among the seasons. Gender and season were significantly associated with *Pfkelch13* mutation (Table 65).

The odds of *Pfkelch13* mutation among participants living in the coastal zone was high compared to those living in the forest zone. The risk of *Pfkelch13* mutation among participants is higher in Abura-Asebu-Kwamankese District (3.30 times more), Agona Swedru Municipality (2.09 times more), Cape Coast Municipal (1.44% times more) and Gomoa East District (3.96% times more) compared to Assin Central Municipality (Table 65). There risk of *Pfkelch13* mutation in *Pfkelch13* among participants living in Abura-Asebu-Kwamankese District and Gomoa East District compared to Assin Central Municipality was statistically significant (Table 65). In the adjusted logistic regression, only the study district had a statistically significant effect on *Pfkelch13* mutation in *Pfkelch13* after adjusting for age, gender, season, and ecological zone (Table 65).

Table 65: Unadjusted and Adjusted Logistic Regression of factors influencing *Pfkelch13* resistance in *Pfkelch13* among participants in the Central Region of Ghana

Explanatory Variables	COR [95% CI]	*P-value*	AOR [95% CI]	*P-value*
Age group				
20 - 59 years	Ref		ref	
Below 5 years	0.57 [0.33-0.99]	0.045*	0.79 [0.42-1.48]	0.455
5 - 9 years	0.72 [0.38-1.33]	0.292	1.03 [0.52-2.03]	0.94
10 - 14 years	0.79 [0.34-1.82]	0.574	0.65 [0.27-1.57]	0.336
15 - 19 years	1.13 [0.55-2.33]	0.736	1.47 [0.69-3.14]	0.322
60 plus years	2.06 [0.61-7.02]	0.243	2.49 [0.69-8.90]	0.162
Gender				
Female	Ref		ref	
Male	0.80 [0.52-1.22]	0.294	0.98 [0.60-1.60]	0.95
Ecology				
Forest zone	Ref		ref	
Coastal zone	1.24 [0.80-1.92]	0.326	1.35 [0.74-2.46]	0.335
Season				
Rainy	Ref		ref	
Dry	0.96 [0.62-1.50]	0.869	1.02 [0.61-1.71]	0.929
Study district				
ACM	Ref		ref	
AAK	3.30 [1.70-6.39]	<0.001**	2.40 [1.11-5.19]	0.026*
ASM	2.09 [0.83-5.26]	0.118	2.14 [0.82-5.62]	0.121
CCM	1.44 [0.80-2.57]	0.223	1	
GED	3.96 [2.16-7.26]	<0.001**	4.13 [2.11-8.07]	<0.001**
Constant	N/A		0.54 [0.32-0.91]	<0.001**

*p<0.05 and p<0.001** were considered statistically significant COR - Crude Odds Ratio; AOR- Adjusted Odds Ratio. Overall Adjusted model: LR Chi-squared (11) = 33.49; *P-value* =0.0004* and R-squared=0.0676. AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality.
Source: Fieldwork, 2021

Exploring factors influencing putative drug-resistant haplotype to *Pfcrt* gene

Table 66 shows factors influencing putative drug-resistant haplotype in the *Pfcrt* gene. In the adjusted model, the odds of participants living in the forest zone relative coastal zone are 0.67 times more likely to report putative drug-resistant haplotype compared to non-putative drug-resistant haplotype after controlling for the effect of age, gender, and season (Table 66). Similarly,

the odds of participants living in the rainy season relative to the dry season are 2.38 times more likely to report putative drug-resistant haplotype in the *Pfcrt* gene compared to non-putative drug-resistant haplotype in the *Pfcrt* gene after controlling for the effect of age, gender and ecological zone (Table 66). The results further showed that the odds of males relative to females are 1.25 times more likely to report putative drug-resistant haplotype in the *Pfcrt* gene compared to non-putative drug-resistant haplotype in the *Pfcrt* gene after controlling for the effect of age, ecological zone, and season (Table 66).

Table 66: Unadjusted and Adjusted Logistic Regression of factors influencing putative drug resistance haplotypes to Chloroquine among participants in the Central Region of Ghana

Explanatory Variables	COR [95% CI]	*P-value*	AOR [95% CI]	*P-value*
Age group				
20 - 59 years	Ref		ref	
Below 5 years	1.61 [0.32-8.13]	0.565	1.61 [0.27-9.44]	0.598
5 - 9 years	0.83 [0.08-8.09]	0.869	0.61 [0.06-6.52]	0.684
10 - 14 years	9.04 [1.90-43.02]	**0.006***	8.84 [1.66-47.17]	**0.011***
15 - 19 years	1	0.442	1	
60 plus years	4.00 [0.39-41.23]	0.244	3.42 [0.31-37.55]	0.315
Gender				
Female	Ref		ref	
Male	1.57 [0.50-4.96]	0.440	1.06 [0.29-3.87]	0.931
Ecology				
Forest zone	Ref		ref	
Coastal zone	1.52 [0.47-4.88]	0.482	1.97 [0.42-9.27]	0.393
Season				
Rainy	Ref		ref	
Dry	1.05 [0.31-3.56]	0.936	1.44 [0.35-5.90]	0.614
Study district				
ACM	Ref		ref	
AAK	2.08 [0.45-9.60]	0.722	0.28 [0.03-2.82]	0.278
ASM	1		1	
CCM	2.17 [0.53-8.94]	0.282	1	
GED	1.29 [0.28-5.91]	0.740	0.99 [0.18-5.65]	0.999
Constant	N/A		0.02 [0.01-0.08]	<0.001**

*p<0.05 and p<0.001** were considered statistically significant COR - Crude Odds Ratio; AOR - Adjusted Odds Ratio. Overall Adjusted model: LR Chi-squared (9) = 10.97; *P-value*=0.2780 and R-squared=0.1058.
Source: Fieldwork, 2021

Exploring factors influencing Putative Pyrimethamine Resistant Haplotype in *Pfdhfr* gene

Table 67 shows factors influencing the putative *Pyrimethamine* resistant Haplotype in *the Pfdhfr* gene. The unadjusted logistic regression shows that age, gender, ecology, season, and study district showed no statistically significant effect on putative *Pyrimethamine* resistant Haplotype in *the Pfdhfr* gene. The analysis revealed that the risk of putative

Pyrimethamine resistant Haplotype in *the Pfdhfr* alleles was low among age-group below 5years [OR=0.37, 95% CI=0.14 - 1.00] and 5 – 9[OR=0.95, 95% CI=0.27 – 3.38] compared to 20 - 59 years respectively. However, the risk was high among 10 – 14 [OR=1.60, 95% CI=0.19 – 13.48] and 15 – 19 years [OR=1.50, 95% CI=0.31 – 7.28] compared to 20 - 59 years respectively (Table 67). The risk of putative *Pyrimethamine* resistant haplotypes in *the Pfdhfr* gene among age-group below 5 years is 34% less compared to 20 - 59 years. However, the risk is comparable between the age group 60 plus years compared to 20 - 59 years respectively (Table 67). The analysis also revealed that the chance of getting putative *Pyrimethamine* resistant haplotypes in *the Pfdhfr* gene among male participants is 54% more compared to female participants. With respect to season, the analysis revealed that the chance of getting putative *Pyrimethamine* resistant Haplotype in *the Pfdhfr* gene among participants in the dry season is 48% less compared to participants in the rainy season (Table 67). The chance of getting the putative *Pyrimethamine* resistant haplotype in *the Pfdhfr* gene among participants living in the coastal zone is 44% less compared to those living in the forest zone. The risk of getting putative *Pyrimethamine* resistant haplotype in *the Pfdhfr* gene among participants is less in Abura-Asebu-Kwamankese District, Agona Swedru Municipality, Cape Coast Municipal and Gomoa East District compared to Assin Central Municipality (Table 67). In the adjusted logistic regression, only season had a statistically significant effect on putative *Pyrimethamine*-resistant Haplotype in *the Pfdhfr* gene after adjusting for age, gender, ecological zone, and study district (Table 67).

Table 67: Unadjusted and Adjusted Logistic Regression of factors influencing Putative Drug Resistant Haplotype to *Pfdhfr* gene

Explanatory Variables	COR [95% CI]	*P-value*	AOR [95% CI]	*P-value*
Age group				
20 - 59 years	Ref		ref	
Below 5 years	0.66 [0.28-1.58]	0.355	0.37 [0.14-1.00]	0.051
5 - 9 years	1.22 [0.38-3.95]	0.742	0.95 [0.27-3.38]	0.94
10 - 14 years	2.06 [0.26-16.67]	0.497	1.60 [0.19-13.48]	0.665
15 - 19 years	1.59 [0.34-7.46]	0.554	1.50 [0.31-7.28]	0.613
60 plus years	1		1	
Gender				
Female	Ref		ref	
Male	1.54 [0.69-3.48]	0.294	1.97 [0.77-5.03]	0.157
Ecology				
Forest zone	Ref		ref	
Coastal zone	0.56 [0.26-1.20]	0.138	0.43 [0.14-1.38]	0.157
Season				
Rainy	Ref		ref	
Dry	0.52 [0.25-1.11]	0.090	0.38 [0.16-0.94]	0.036*
Study district				
ACM	Ref		ref	
AAK	0.36 [0.12-1.05]	0.062	1.01 [0.29-3.55]	0.985
ASM	0.71 [0.14-3.55]	0.674	0.89 [0.16-4.78]	0.888
CCM	0.59 [0.20-1.79]	0.356	1	
GED	0.68 [0.24-1.94]	0.468	0.40 [0.12-1.27]	0.121
Constant	N/A		26.05 [9.13-74.32]	<0.001**

*p<0.05 and p<0.001** were considered statistically significant
COR - Crude Odds Ratio; AOR - Adjusted Odds Ratio
Overall Adjusted model: LR Chi-squared (11) = 8.58; *P-value*=0.6606 and R-squared=0.0416, AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality
Source: Fieldwork, 2021

Exploring factors influencing putative Sulfadoxine resistant Haplotype in *Pfdhps*

Table 68 shows factors influencing putative sulfadoxine-resistant Haplotype in *the Pfdhps* gene. The unadjusted logistic regression shows that age, gender, ecology, season, and study district showed no statistically

significant effect on the putative *Sulfadoxine* resistant Haplotype in *the Pfdhps* gene. The analysis revealed that the odds of getting putative *Sulfadoxine* resistant Haplotype in *the Pfdhps* gene was high among age-group below 5years, 5 – 9 and 15 – 19 years is [OR=1.60, 95% CI=0.32 - 8.04],[OR=3.56, 95% CI=0.0.34– 36.91] and [OR=1.95, 95% CI=0.20 – 18.70] more compared to 20 - 59 years respectively (Table 68). The odds were comparable among age-group 10 – 14 and 60 plus years compared to 20 - 59 years respectively (Table 68). The analysis also revealed that the odds of putative *Sulfadoxine* resistant haplotypes in *the Pfdhps* gene among male participants was less compared to female participants. With respect to season, the analysis revealed that the chance of getting the putative *Sulfadoxine* resistant Haplotype in *the Pfdhps* gene among participants in the dry season is low compared to participants in the rainy season (Table 68). The risk of being infected the putative *Sulfadoxine* resistant haplotype in *the Pfdhps* gene among participants living in the coastal zone is high compared to those living in the forest zone. The chance of getting putative *Sulfadoxine* resistant Haplotype in *the Pfdhps* gene among participants is more in Abura-Asebu-Kwamankese District (150% more), Cape Coast Municipal (238% more), and Gomoa East District (128% more) compared to Assin Central Municipality (Table 68). However, the odds were comparable among participants in Agona Swedru Municipality compared to Assin Central Municipality (Table 68). In the adjusted logistic regression, age had no statistically significant effect on the putative *Sulfadoxine* resistant haplotype in *the Pfdhps* gene after adjusting for gender, ecological zone, and study district. Similarly, other exploratory variables such as gender, ecological zone, season, and study district had no

statistically significant influence on putative *Sulfadoxine* resistant Haplotype in *the Pfdhps* gene in the adjusted model (Table 68).

Table 68: Unadjusted and Adjusted Logistic Regression of factors influencing Putative Sulfadoxine Resistant Haplotype to *Pfdhps*

Explanatory Variables	COR [95% CI]	*P-value*	AOR [95% CI]	*P-value*
Age group				
20 - 59 years	Ref		ref	
Below 5 years	0.77 [0.20-2.97]	0.708	1.60 [0.32-8.04]	0.571
5 - 9 years	1.99 [0.23-17.50]	0.534	3.56 [0.34-36.91]	0.288
10 - 14 years	1		1	
15 - 19 years	1.40 [0.16-12.39]	0.763	1.95 [0.20-18.70]	0.563
60 plus years	1		1	
Gender				
Female	Ref		ref	
Male	0.51 [0.15-1.71]	0.275	0.38 [0.09-1.56]	0.178
Ecology				
Forest zone	Ref		ref	
Coastal zone	2.02 [0.43-9.55]	0.373	2.90 [0.33-25.40]	0.337
Season				
Rainy	Ref		ref	
Dry	0.84 [0.24-2.93]	0.780	0.67 [0.17-2.63]	0.561
Study district				
ACM	Ref		ref	
AAK	2.50 [0.30-20.96]	0.398	0.77 [0.04-13.81]	0.862
ASM	1		1	
CCM	3.38 [0.40-28.13]	0.261	1	
GED	2.28 [0.46-11.29]	0.312	2.28 [0.38-13.76]	0.370
Constant	N/A		18.00 [4.65-69.71]	<0.001**

*p<0.05 and p<0.001** were considered statistically significant
COR - Crude Odds Ratio; AOR - Adjusted Odds Ratio
Overall Adjusted model: LR Chi-squared (9) = 5.88; *P-value* = 0.7522 and R-squared= 0.0646, AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality
Source: Fieldwork, 2021

Exploring factors influencing Putative Drug Resistant Haplotype in *Pfmdr 1* gene

Table 69 shows factors influencing putative drug-resistant Haplotype in *Pfmdr 1* gene. The unadjusted logistic regression shows that only season has a statistically significant effect on putative drug-resistant Haplotype in *Pfmdr 1* gene. Age, gender, ecology, and study district showed no statistically

significant influence on putative drug-resistant Haplotype in *Pfmdr 1* gene (Table 69). The analysis revealed that the odds of getting putative drug-resistant haplotype in *the Pfmdr 1* gene was low among age-groups below 5years [OR=0.97, 95% CI=0.45 - 2.07], 5 – 9 years[OR=0.82, 95% CI=0.36– 1.84] and 15 - 19 years [OR=0.94, 95% CI=0.37 – 8.81] compared to 20 - 59 years respectively (Table 69).however, the odds were high among age-group below 10 - 14[OR=1.24, 95% CI=0.38– 4.05], and 60 plus years[OR=1.83, 95% CI=0.38– 8.81] compared to 20 - 59 years respectively. The analysis also revealed that the risk of getting putative drug-resistant haplotype in *the Pfmdr 1* gene among male[OR=1.30, 95% CI=0.72– 2.35] participants is high more compared to female participants. Similarly, the risk of getting putative drug-resistant haplotype in *the Pfmdr 1* gene among participants living in the coastal zone [OR=1.18, 95% CI=0.55– 2.53] was high compared to those living in the forest zone. The analysis also revealed that the risk of getting putative drug-resistant haplotype in *the Pfmdr 1* gene among participants in the dry season [OR=0.67, 95% CI=0.37– 1.24] was lower compared to participants in the rainy season (Table 69). The chance of getting putative drug-resistant Haplotype in *Pfmdr 1* gene among participants is higher in Abura-Asebu-Kwamankese District and, and Gomoa East District compared to Assin Central Municipality (Table 69). The of is similar in Cape Coast Municipal compared to Assin Central Municipality. However, the chance of getting putative drug-resistant Haplotype in *the Pfmdr 1* gene among participants is less in Agona Swedru Municipality was lower compared to Assin Central Municipality. In the adjusted logistic regression, the study district had no statistically significant effect on putative drug-resistant

Haplotype in *Pfmdr 1* gene after adjusting for age, gender, season, and ecological zone. Similarly, other explanatory variables had no statistically significant influence on putative drug-resistant Haplotype in *Pfmdr 1* gene in the adjusted model (Table 69).

Table 69: Unadjusted and Adjusted Logistic Regression of factors influencing Putative Drug Resistant Haplotype in *Pfmdr* 1 gene among participants in the Central Region of Ghana

Characteristics	COR [95% CI]	*P-value*	AOR [95% CI]	*P-value*
Age group				
20 - 59 years	Ref		ref	
Below 5 years	1.06 [0.55-2.06]	0.858	0.97 [0.45-2.07]	0.933
5 - 9 years	0.88 [0.42-1.86]	0.743	0.82 [0.36-1.84]	0.629
10 - 14 years	1.40 [0.44-4.42]	0.570	1.24 [0.38-4.05]	0.724
15 - 19 years	0.88 [0.36-2.17]	0.785	0.94 [0.37-2.36]	0.888
60 plus years	1.76 [0.37-8.33]	0.473	1.83 [0.38-8.81]	0.453
Gender				
Female	Ref		ref	
Male	1.25 [0.74-2.14]	0.403	1.30 [0.72-2.35]	0.384
Ecology				
Forest zone	Ref		ref	
Coastal zone	1.04 [0.59-1.81]	0.902	1.18 [0.55-2.53]	0.665
Season				
Rainy	Ref		ref	
Dry	0.59 [0.35-1.01]	0.053*	0.67 [0.37-1.24]	0.204
Study district				
ACM	Ref		ref	
AAK	1.09 [0.48-2.44]	0.838	1.04 [0.40-2.72]	0.941
ASM	0.64 [0.26-1.56]	0.328	0.75 [0.29-1.95]	0.549
CCM	1.18 [0.57-2.45]	0.655	1	
GED	1.77 [0.83-3.78]	0.137	1.50 [0.66-3.42]	0.339
Constant	N/A		3.31 [1.78-6.17]	<0.001**

*p<0.05 and p<0.001** were considered statistically significant.
COR - Crude Odds Ratio; AOR - Adjusted Odds Ratio.
Overall Adjusted model: LR Chi-squared (11) = 7.71; *P-value* = 0.7389 and R-squared=0.0216, AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality. Source: Fieldwork, 2021

Exploring factors influencing Putative Drug Resistant Haplotype to *Pfdhfr/Pfdhps* mutations

Table 70 shows factors influencing putative drug-resistant Haplotype to *Pfdhfr/Pfdhps* mutations. The unadjusted logistic regression shows that age, gender, ecology, season, and study district showed no statistically significant effect on putative drug-resistant Haplotype to *Pfdhfr/Pfdhps* mutations. The analysis revealed that the odds of being infected with a putative drug-resistant haplotype to *Pfdhfr/Pfdhps* mutations was high among age-group below 5[OR=1.02, 95% CI=0.33 – 3.13], 5 – 9[OR=1.45, 95% CI=0.41– 5.10]. 10 – 14[OR=1.15, 95% CI=0.23– 5.86], 15 – 19[OR=1.31, 95% CI=0.33– 5.15], and similar among age group 60 plus years compared to 20 - 59 years (Table 70). The analysis also revealed that the chance of being infected with putative drug-resistant haplotype to *Pfdhfr/Pfdhps* mutations among male participants [OR=0.91, 95% CI=0.39– 2.15] is lower compared to female participants and in the coastal ecology [OR= 0.46, 95% CI=0.15– 1.35] compared to the forest ecology. With respect to season, the analysis revealed that the chance of getting putative drug-resistant Haplotype to *Pfdhfr/Pfdhps* mutations among participants in the dry season [OR=1.39, 95% CI=0.53– 3.63]is high compared to participants in the rainy season (Table 70). The odds of getting putative drug-resistant haplotype to *Pfdhfr/Pfdhps* mutations among participants is high in Abura-Asebu-Kwamankese and Gomoa East Districts compared to Assin Central Municipality (Table 70). The odds were however lower in the Agona Swedru Municipality (64% less) and comparable in the Cape Coast Metropolis compared to Assin Central Municipality (Table 70). In the adjusted logistic regression, age had no statistically significant effect on putative drug-resistant

Haplotype to *Pfdhfr/Pfdhps* mutations after adjusting for gender, ecological zone, season, and study district. Similarly, other explanatory variables such as gender, ecological zone, season, and study district had no statistically significant influence on putative drug-resistant Haplotype to *Pfdhfr/Pfdhps* mutations in the adjusted model (Table 70).

Table 70: Unadjusted and Adjusted Logistic Regression of factors influencing putative drug resistance haplotypes to SP among participants in the Central Region of Ghana

Characteristics	COR [95% CI]	*P-value*	AOR [95% CI]	*P-value*
Age group				
20 - 59 years	Ref		ref	
Below 5 years	1.11 [0.42-2.96]	0.828	1.02 [0.33-3.13]	0.975
5 - 9 years	1.23 [0.38-4.03]	0.730	1.45 [0.41-5.10]	0.558
10 - 14 years	1.03 [0.21-5.01]	0.974	1.15 [0.23-5.86]	0.867
15 - 19 years	1.19 [0.31-4.48]	0.801	1.31 [0.33-5.15]	0.699
60 plus years	1		1	
Gender				
Female	Ref		ref	
Male	0.90 [0.41-1.96]	0.784	0.91 [0.39-2.15]	0.835
Ecology				
Forest zone	Ref		ref	
Coastal zone	0.83 [0.37-1.87]	0.655	0.46 [0.15-1.35]	0.156
Season				
Rainy	Ref		ref	
Dry	1.26 [0.54-2.98]	0.593	1.39 [0.53-3.63]	0.504
Study district				
ACM	Ref		ref	
AAK	1.50 [0.31-7.24]	0.610	2.98 [0.54-16.51]	0.211
ASM	0.36 [0.10-1.31]	0.122	0.34 [0.09-1.32]	0.119
CCM	0.51 [0.19-1.38]	0.185	1	
GED	0.95 [0.31-2.93]	0.931	1.06 [0.31-3.59]	0.930
Constant	N/A		8.57 [3.39-21.67]	<0.001**

*p<0.05 and p<0.001** were considered statistically significant
COR - Crude Odds Ratio; AOR - Adjusted Odds Ratio; SP - Sulfadoxine-pyrimethamine
Overall Adjusted model: LR Chi-squared (10) = 5.33; *P-value* = 0.8618 and R-squared=0.0294,
AAK - Abura-Asebu-Kwamankese District, ASM - Agona Swedru Municipality, CCM - Cape Coast Metropolis, GED - Gomoa East District, ACM - Assin Central Municipality
Source: Fieldwork, 2021

Discussion

Malaria affects Ghana and all sub-Saharan African countries where the disease is of public health importance. The endemicity, severity, and disease outcomes of malaria results from the interaction between the host, parasite strain, and its environment leading to unstable malaria transmission patterns (Abate et al., 2022; Mitchell et al., 2022). This study looked at the prevalence of malaria and the *Plasmodium* species. This was to assess the disease burden and the parasite population. Simultaneously, the genetic dynamics and diversity of the parasite in the forest and coastal ecological niches together with measures towards the control and elimination of this disease hinder case management (Sondo et al., 2019).

The Central Region of Ghana is made up of diverse ecological hubs, however, the two main ecological zones are the forest and coastal zones. Each of these zones has its eco-geographic characteristics, even though they experience common seasonal climate patterns of varied duration. Such environmental differences may require peculiar strategic control intervention to achieve the reduction in burden of malaria. Thus, this study investigated the prevalence and molecular epidemiological trends in participants attending selected health facilities in the region, considering seasonal and ecological impact to understand the contextual diversity within each study site. Furthermore, the sociodemographic and environmental risk variables influencing malaria occurrence were evaluated. In most malaria-endemic countries, *P. falciparum* is the major parasite and represents close to 95% of the total malaria parasite population (WHO, 2020). Previous reports on the distribution of non-falciparum malaria parasites in Ghana vary across different

regions of the country (Ejigu & Wencheko, 2021). However, to date, there have been no reports of *P.* vivax in the country and in the Central region (Obboh et al., 2020). According to the 2018 world malaria report, *P. falciparum* represents 100% of the parasite population in Ghana (Amoah et al., 2019), thus skewing the focus of this study to investigate the molecular diversity of *P. falciparum* in the Central Region of Ghana. *P. falciparum* infections in endemic areas are said to be of multiple clones (Lo et al., 2017; Sondo et al., 2019; Touray, 2020 ; Wong, Wenger, Hartl, & Wirth, 2018).

Secondly, this study investigated the dynamics of *P. falciparum* infections in two ecological zones of the Central Region. This was by studying the genetic diversity through the description of the Complexity of Infection in *P. falciparum* (WHO, 2020, 2021). Currently, drug resistance to all antimalarials has been observed through the development of SNPs (Amaratunga et al., 2016). It is, therefore, necessary to report all observed SNPs, particularly developed to the most recent antimalarial drugs. The *Pfkelch-13* gene has been reported as responsible for resistance to Artemisinin combinations (ACTs) in South East Asia, however, molecular markers for resistance in Africa are unknown (Matrevi et al., 2022). Thus, this study investigated the prevalence of SNPs associated with antimalarial drug resistance in the region. The study analysed the genotype alleles in *Pfcrt amino acid codons* C72, V73, M74I, N75E, K76T, *Pfmdr1* (N86Y, Y184F, D1246Y), *Pfdhps* (S436F, A437G, K540E, A581G, A613S), *Pfdhfr* (N51I, C59R, S108N, I164L) and *Pfkelch13*gene to determine the molecular characteristics of *P. falciparum* infections in the Central Region of Ghana. The

fourth objective was to investigate the risk factors associated with drug-resistant haplotypes among the study participants.

Evidence drawn from prevalence studies indicates that it is an affordable means for assessing malaria control measures and programmes at local and national levels (Umaru & Uyaiabasi, 2015). This study used Rapid Diagnostic Tests (RDT) and microscopy to determine the prevalence of malaria parasites in the study sites. The prevalence observed by microscopic examinations was (38.19%) whilst that by RDT was 61.8% in the current study. However, the microscopic malaria prevalence in the present study was higher compared to other studies conducted in the regional capital, Cape Coast - 24% (Mensah et al., 2021) and in the Eastern region of Ghana - 32% by microscopy and 33% by RDT (Amoah et al., 2019). The low prevalence recorded by these two studies may be due to the asymptomatic and afebrile study cohort involved in their study. Also, the number of districts in the current study (5 districts) compared to Mensah's (1 district) study may account for such variations. Another study in Northern Ghana recorded a 21.6% prevalence by microscopy, however, his study involved pregnant women only (Ahenkorah et al., 2020). In a cross-sectional study among symptomatic individuals in 10 healthcare facilities in Ghana, parasite prevalence rates were 32.3% (6266/19402) by RDT and 16.0% (2984/18616) by microscopy (Abuaku et al., 2021). The difference may lie within the methodology used in arriving at the prevalence. The current study shows a regional prevalence whilst Abuaku and his colleagues reports a cumulative average recorded from different regions in the country. Notwithstanding, this study is comparable to studies in the Ashanti region where the molecular

prevalence of *Plasmodium* species was 73% (Heinemann et al., 2020). The high prevalence among asymptomatic participants in the Ashanti region may be because the study site, Asante Akim North district, lies in the rainforest belt, which provides favourable eco-geographic conditions for both parasites and vectors.

Outside Ghana, varying prevalence have equally been observed: 32.54% in Gabon (Mba et al., 2022), 36.57% in the Central African Republic (Doutoum et al., 2019), 29 % for *P. falciparum* in Ethiopia (Negatu et al., 2022), 47.9% in Congo (Singana et al., 2019), and 55 % and 64.8% in Nigeria (Awosolu et al., 2021; Bajoga et al., 2019), 74% in Kenya (Touray, 2020) and 60.4 % in Gabon (Boukoumba et al., 2021). Variability in the prevalence of malaria among different study settings was also reported in the villages in Kokap (Lestari et al., 2020), and in over twelve years in Ethiopia (Haileselassie et al., 2022). Variations in the time of the studies might also be responsible for the differences in the prevalence of malaria within study sites, seasons, and eco-geographical settings as supported by global and national reports (Abate et al., 2022; Abuaku et al., 2021; WHO, 2021). The differences can also be justified by the study population, and the variations in the local epidemiology of the disease. Similarly, the extent of implementation of malaria intervention activities may account for the disparities recorded. In addition, the sensitivity and specificity of the investigative techniques employed in each study may also affect the result outcome (Mba et al., 2022).

Microscopic examination of blood smears did not determine any other species apart from *P. falciparum* whereas analysis of *Plasmodium* DNA sequences identified *P. falciparum* (98.8%). *Plasmodium* co-infections of *P.*

falciparum and *P.ovale* as well as *P.falciparum, P. vivax* and *P.malariae* accounted for 0.8 % and 0.4% respectively. This was expected as gene sequencing is of high specificity and sensitivity (Fitri et al., 2022) compared to microscopy. The high prevalence of *P. falciparum* infections was confirmed by the current study and by the 98.0% prevalence of infections recorded by Abuaku (Abuaku et al., 2021). Similar studies found 95.9% *P. falciparum* and 4.1% being *P. malariae* infections in the Central Region (Obboh et al., 2020). In the Volta Region of Ghana, 87.3% *P. falciparum* and 12.7% *P. malariae* were reported among malaria-positive patients (Sakzabre et al., 2020).The differences may be due to the endemicity of parasites in different eco-geographical hub in the Central region and Ghana at large. The findings of the current study is similar to results found in Kenya, where microscopy identified only *P. falciparum* infections, whereas RT-PCR identified *P. malariae* and *P. ovale* mono-infections and co-infections with *P. falciparum* (Otambo et al., 2022). *Plasmodium* coinfection showed a similar pattern of triple infection of *P. falciparum, P. malariae* and *P. vivax* in another study in Nigeria (Oboh et al., 2022). Different prevalence's of *P.falciparum* have been recorded at 95% of malaria infections in Burkina Faso (Yaro et al., 2021) and low *P. falciparum* prevalence (4% infection) in Pakistan (Hussain et al., 2021). Researchers recorded a 29% *P. flaciparum* in Ethiopia (Negatu et al., 2022), 1.3% in Pakistan (Khan, Rahman, Shafiq, Ihsan, & Khan, 2019) and 19.2% in Tanzania (Mitchell et al., 2022). In these areas however, *P. vivax* was of high prevalence compared to *P. falciparum.* Nevertheless, another study found a 55% prevalence of *P. vivax* whereas 45% was recorded for *P. falciparum* (Surve, Kulkarni, Rathod, & Bindu, 2017).

This study highlights the detection of *P. vivax* in very low frequency and contradict reports of no *P. vivax* infections in Ghana (Amoah et al., 2019; Brown et al., 2021) and Central region (Obboh et al., 2020). However, the finding of *P. vivax* adds to the growing concern about *P. vivax* among individuals in Sub-Saharan Africa (SSA) where it was thought to be initially non-existent (Oboh et al., 2022; Twohig et al., 2019). Some studies that have reported the presence of *P. vivax* in their region include: 15% *P. vivax* prevalence in Benin by PCR (Poirier et al., 2016). However, a high seropositive (53%) and 8% PCR-positive samples were of *P. vivax* in Kedougou, south-eastern Senegal (Niang et al., 2017). In Cameroon, prevalence's of 35%, 0.5% and 2% were recorded among isolates in Dschang, Santchou and Kye´-ossi respectively (Dongho et al., 2021). The disparities in species prevalence lie in the differences in the geography of study sites, vector prevalence, and the large asexual count of *P. falciparum,* which prevents a further probe for other species in the likely event of a coinfection during microscopic examination (Abate et al., 2022). The detection of *P. vivax* may be a result of climatic change resulting from climatic conditions which favour the survival of the species in different ecological settings. For example, global warming can cause latitudinal and altitudinal shifts in vector distributions, thereby altering the species prevalence in a given geographic area (Xia et al., 2020). Similarly, this may be due to the presence of an unknown mosquito vector in the Central Region as suggested by Dongho and his colleagues in Cameroon (Dongho et al., 2021). On the other hand, *P. vivax* could have been by importation by a person from a high *P. vivax* endemic area as the Central

region has high accretion rate of foreign tourists because of its historical cultural heritage.

In the current study, the risk of contracting malaria in all the study districts where found to be statistically similar with ORs ranging from 0.52 to 2.47 in the unadjusted logistic regression, and 0.28 to 2.36 in the adjusted models. In another study, in Kenya, univariate analysis showed that residency in the lakeshore zone was associated with an increased risk of sub microscopic malaria infection whilst multivariate analysis revealed a significant association (Otambo et al., 2022). Even though children under 5 years are said to be the most vulnerable group to malaria infection (WHO, 2018), this age group did not have the highest prevalence in this study.

The most infected age group to malaria was 20-59 years category. A similar finding was reported where the 15-49-year group was most affected by malaria in Franceville, Gabon (Mba et al., 2022). The justification for this finding is that majority of study participants fell within this age group in the current study. Also, this age group is considered the productive age and as a result of occupational hazards may frequently be engaged in outdoor activities. As expected, comparing all age groups below 19 years of age, the age group less than 5 years was most infected with malaria in the study. This finding is consistent with the report of the World health organisation, the Sub-Saharan sub-region (Awosolu et al., 2021; Bajoga et al., 2019; WHO, 2020, 2021), and in most studies conducted in Ghana (Adjah et al., 2018; Tandoh et al., 2021) that found high malaria prevalence in children under 5 years due to the underdeveloped protective immunity against malaria.

This study also found that the odds of getting malaria varied among the different age groups in the districts. However, there was a high risk of malaria infection in the age group 5-9 years compared with all the other age groups across all sample sites, the unadjusted (OR= 1.61; *P- value* <0.011) and adjusted (OR= 2.14; *P-value* <0.001). Nevertheless, in a study in the eastern and Central region of Ghana, the age group 12–14 years had more asymptomatic infections than those aged 6–8 years (OR = 1.28, *P-value* = 0.005) (Mensah et al., 2021).

Ghana showed interest in 2016 and was selected in 2017 to be a part of the Malaria Vaccine Pilot Implementation Project (MVIP) of the RTS, S/AS01E malaria vaccine (MOH., 2016). Ghana participated in Phase 3 clinical trials where children aged 6 weeks to 17months were immunized with four doses of the vaccine at ages: 0, 1, and 2 months and a booster dose at 20 months (Asante, Adjei, Enuameh, & Owusu-Agyei, 2016). The Phase 3 clinical trials involved approximately 15,000 infants and demonstrated the efficacy and safety of the vaccine which is purposed to prevent the *P. falciparum* species from infecting red blood cells. The vaccine was reported to reduce the disease burden of malaria by approximately 40% when used in conjunction with other malaria preventive measures, such as bed nets and insecticides (Adeshina, Nyame, Milner, Milojevic, & Asante, 2022; Asante et al., 2016). Thus, the low prevalence among the age group less the 5 years can also be attributed to the fact that children in this category in the Central Region of Ghana formed a part of the malaria vaccine (RTS, S/AS01E) trial.

A shift in the prevalence in this study, is also in concordance with the prevalence of malaria among children under 5 years as reported in Ethiopia

and The Gambia (Abate et al., 2022; Collins et al., 2022). A recent study recorded an 18% prevalence among children under 5 years and 25% among children aged 5–9 years in Senegal (Ndiaye et al., 2019). However, in Mali, there has been a report on the increasing malaria prevalence among children older than 10 years while children aged 6 months–5 years were least infected (P = 0.026) (Coulibaly et al., 2021). High malaria prevalence in the age group 6–10 years compared to children under 5 years has been reported in Nigeria from the Bivariate Logistic Regression analysis in their study (Awosolu et al., 2021). Malaria prevalence among age group greater than 50 years has been documented in China (Xia et al., 2020) and above age group 60 years in Nigeria (O.R=1.35) (Bello, 2021). The shift in age burden from age group below five years to age group 5-9years may be due to the efficacy of control measures, such as indoor residual spraying (IRS), long-lasting insecticide-treated nets (LLINs) as well as measures such as prompt diagnosis (RDT) and treatment than the older age groups.

Generally, the results of the present study indicated that malaria was prevalent in all age groups. However, the bivariate analysis showed an association between malaria and infection and age group below five (5 years) in both forest and coastal zones. The conformity in both ecological zones in this age group may be due to the underdeveloped protective immunity among children less than 5 years of age, which makes them vulnerable to infection (Afutu et al., 2021; Allotey et al., 2021; Osarfo, Ampofo, & Tagbor, 2022). Other age groups 5-9 years and 60 years plus were also associated with malaria in the forest zone but not in the coastal zone. Malaria in the age group 5-9 years may be due to lower ITN use, as this age group is not a beneficiary

of this control intervention in the study areas. Also, there is very little parental supervision among this age group as observed in the study sites which can account for the high prevalence and association with malaria infection due to outdoor biting. However, an association of malaria with the age group 60 years plus can be linked to the compromised immunity among this age group.

Among the seasons, malaria infection was associated with the age group below 5 years and age group 5-9 years in the rainy season, whilst the age group 60 years plus was associated with malaria in the dry season. This may be due to the favourable wet conditions in which *Plasmodium* thrives. Thus, vector densities in the rainy season were sufficiently high to maintain high transmission in the forest zones than in the coastal zone. Also, higher gametocyte prevalence previously observed in dry season along the coast of the Central Region (Ayanful-Torgby et al., 2018) could have sustained infections in this age group.

In the current study, females (1153,75.6%) were most burdened with malaria compared to their male (372, 24.3%) counterparts. The situation was the same when broken down into the study districts. However, males had a higher odd of contracting malaria, although not statistically significant (P-value = 0.539). Thus, gender did not influence the risk of malaria within the study sites. In other studies females accounted for 69.07% of malaria in Ghana (Sakzabre et al., 2020), 70% of the study population in Uganda (Okiring et al., 2022), and 58.91% in Gabon (Mba et al., 2022). However, a cross-sectional survey in Ghana reported an almost equal prevalence in gender - female (1986, 76.7%) and males (1669, 72.5%) (Mensah et al., 2021), whilst another study in Ghana reports that female participants were less likely to have

malaria parasites either by microscopy (22.0%) or RDT (30.0%) compared with males (Abuaku et al., 2021). Low odds of infection in females compared to males also agrees with the lower odds among female participants compared with males in Abuaku study (Abuaku et al., 2021). Similar findings of higher risk of infection in men by 18% than in women in asymptomatic malaria infection have also been reported (Mensah et al., 2021).

Two separate studies reported a high prevalence among the male gender compared to the female gender in their study areas. The study by Awosolu recorded a 60.2% prevalence in males and 50.9% in females in Ibadan, Nigeria (Awosolu et al., 2021) whereas 55% males and 45% females were recorded among three sentinel sites in Kano, Enugu and Plateau states, also in Nigeria (45%) (Ajogbasile et al., 2022). A similar finding, indicated 64.8% of males and 35.2% of females were infected with malaria in Ethiopia (Abate et al., 2022). This result contradicts that obtained in a previous study that observed that women were 2.261 times more likely to be infected with malaria than men (Negatu et al., 2022), Also, the current study agrees with reports from Kenya reporting that females were less likely than males to develop sub microscopic infection (Otambo et al., 2022).

The disparities in the sex pattern of infection in this study compared to other studies could also be attributed to gender-based activities influenced by the culture of the people in the study area. However, the prevalence recorded in the present study is more likely due to the high participation of women in the study. Notwithstanding, the findings suggest that females may be more prone to the disease than males and can attract interventions targeted toward female-biased activities. From observation, females usually get involved in

outdoor activities such as hawking and food vending which makes them stay late outside exposing them to high mosquito bites more than males. Also, the low prevalence in males may be due to their preference for home treatment and self-initiated medication as reported by other studies elsewhere (Quaresima et al., 2021).

In the current study, however, there is a significantly higher risk of women attending Antenatal clinic (ANC) getting malaria infection compared to their outpatients and in-patient counterparts. This may be due to compromised immunity compared with non-pregnant women (Chaponda et al., 2021; Völker et al., 2017), and also influencing the high prevalence of women in the current study. This finding also suggests that irrespective of the vital roles of women in foetal, neonatal, and child care (children under 5 years of age), they do not benefit from malaria interventions targeted at this age group.

Data from this study reveals a high disease burden in the rainy season (71.41%) than in the dry season (28.59%). The results obtained compare with studies that report more malaria cases in the wet than the dry season in Nigeria (Bajoga et al., 2019; Segun et al., 2020). Similar findings have been reported in Burkina Faso with greater malaria intensity after the minor and major rainy seasons (Haileselassie et al., 2022), and in Kokap, Yogyakarta where malaria prevails all year round but predominantly in the rainy (109) season than in the dry season (77) (Lestari et al., 2020). Also, the 2018 world health organization report supports this finding stating that malaria transmission is seasonal, with the peak during and just after the rainy season (WHO, 2018a). Nevertheless, a recent study in Cape Coast reported a 72% prevalence of children harbouring

asexual parasites with gametocytes in Cape Coast during the dry season compared to 59% out of 39 children recorded during the rainy season (Ayanful-Torgby et al., 2018). Similar results were observed in a study in Mali which recorded a high prevalence of infection in the dry season (Coulibaly et al., 2021). However, in Kenya, sub microscopic infections were significantly different across seasonality with high infection in the dry season of November 2019 (19.7%, n = 456), followed by the rainy season of June 2020 (13.9%, n = 458), then the rainy season of June 2019 (12.9, n = 458), and the dry season of November 2020 (10.5%, n = 475) (Otambo et al., 2022). It must be noted that this study was done during minor rains and not during heavy rains. Thus the results of the current study could be due to the adequate availability of pockets of stagnant water in ponds and ditches and gutters particularly around homes increasing the breeding of mosquitoes and disease transmission during the rainy seasons or due to high temperatures shown to inhibit the developmental stages of the parasite and its vectors as reported by other studies elsewhere (Akpan, Adepoju, Oladosu, & Adelabu, 2018; Segun et al., 2020).

The regression modelling indicates a higher odd in the dry season compared to the rainy season. This result compares with studies recording no association between season and total malaria case number in Kokap (*P-value*-0.316) (Lestari et al., 2020), and in Ethiopia (F3,8: 1.982, P:0.195) (Haileselassie et al., 2022). However, the result does not agree with a study in Nigeria that report a positive relationship between rainfall among other climatic conditions with malaria incidence in Abuja (Segun et al., 2020).

The samples for this study were also collected from two distinct ecological zones in the region. In total, malaria found in the forest ecology was significantly more than malaria in the coastal sites (71.02% vs 28.98%). This compares with a recent and similar study in Ghana that reported that asymptomatic malaria prevalence was higher within the forest ecological niche compared to coastal areas of Ghana (Mensah et al., 2021), and among asymptomatic school children in the Sahel and forest zones than the coastal zone (Amoah et al., 2021). Also, a study in India reports the low prevalence of malaria among all eight coastal districts studied compared to the other study sites (Pradhan & Meherda, 2019). The high reports of malaria predominantly in the forest ecological zone may be due to favourable environmental factors such as temperature buffering, humidity, abundant rainfall and the vegetative cover (Mensah., et al., 2021; Mohammed et al., 2022) . Additionally, the presence of multiple streams in the forest ecosystems and the burden of illegal mining have resulted in large pools of water suitable for malaria vector breeding and thus, leading to high transmission (Allotey et al., 2021; Ranjha & Sharma, 2021).

The low prevalence of *P. falciparum* in the coastal zone compared to the forest zones might be due to the high salinity of the water bodies which do not support mosquito breeding. Additionally, *Anopheles melas* are dominant in coastal zones. This species of Anopheles does not support the transmission of malaria parasites (Forson et al., 2022; Hinne, Attah, Mensah, Forson, & Afrane, 2021). Also, in this study, the unadjusted logistic regression indicated that the ecology impacted the risk of malaria even though there was a lower risk of being infected with malaria in the coastal zone. Similarly, Mensah

reported increased odds of asymptomatic malaria in the forest ecological zone (Mensah et al., 2021). A study in India also concluded that Clusters of malaria cases are closely associated with dense forest cover in Southeast Asia (Ranjha & Sharma, 2021). Whilst in a spatiotemporal distributed lag modelling of multiple *Plasmodium* species in a malaria setting of the Greater Mekong Sub-region, the authors concluded that environmental and climatic factors affect the developmental stages of both mosquitoes, and parasites within the mosquitoes (Rotejanaprasert et al., 2021) this observation in their study may be a reason for the disparities within the different ecozones in the current findings.

Various studies have shown that malaria-endemic settings are usually characterized by infections with multiple clones, which directly influence parasite genetic diversity than in low endemic areas (Lo et al., 2017; Sondo et al., 2019; Touray, 2020 ; Wong et al., 2018). Genetic diversity results from the recombination of alleles during the sexual stage in the mosquito vector. Thus, a variety of allelic forms of the parasite are transferred to the susceptible host during a blood meal. Therefore, the genetic diversity of clones per infection can reflect the dynamics of parasitic transmission, where higher transmission yields a higher rate of recombination and vice versa (Huang et al., 2016). With this premise, the current study investigated the prevalence of parasite clones infecting persons in the study area as an indicator of transmission intensity in the ecological zones.

The current study showed that the genetic diversity of isolates was high and ranged from 1 to 6 genotypes per infection. This is high when compared to another study in Ghana that recorded a diversity ranging from 1

to 4 genotypes per infection among 10 sentinel sites in the ten regions of Ghana (Matrevi et al., 2019). Another study using amplicon deep sequencing equally found 1 to 6 clones per infection in China (He et al., 2021). The disparities in genetic diversity may be due to the diverse ecological niches which support the survival of both vector and parasites and confirms the importance of local epidemiologic factors in malaria transmission. Also, the type of participants, time of sampling, and type of samples used may impact the disparities as the use of archived samples may not be representative of current situation in the populace. The present study observed polyclonal infections at a prevalence of 55.5%. Analyzing DNA sequences from the Pf3k data repository of the Malaria Genomic Epidemiology Network (MalariaGEN), Tandoh and his colleagues, found a similar rate of 56% among *Plasmodium* isolates (Tandoh et al., 2021). The prevalence of polyclonal infection also corroborates with findings on parasite diversity in other parts of Ghana, which concluded that *P. falciparum* parasite infections in southern Ghana were diverse. This was after analyzing malaria-positive samples from Obom and Asutsuare (Abukari et al., 2019). In another study in Eswatini, 67% of isolates were polyclonal infections (Roh et al., 2019) whereas 86% polyclonality was found in Congo (Singana et al., 2019). In Yombo, Tanzania and the Zanzibar archipelago, about 50% of the isolates were monoclonal (complexity of Infection is 1) whilst the other half were polyclonal (COI > 1) (Morgan et al., 2020).

The overall mean multiplicity of infection (MOI) of the current study population (the average number of distinct parasite genotypes concurrently infecting a patient) corroborates with other studies in high malaria

transmission areas like Congo – 2.64 (Singana et al., 2019) and 2.38 when comparing AMA1 amino acid frequencies in the DRC (Miller et al., 2017), 2.2 in China (He et al., 2021) and 2.2 in Eswatini (Roh et al., 2019). However the COI was found to be lower compared to the 3.39 observed in Kenya (Touray, 2020) and 3.1 recorded in other parts of Ghana (Lamptey et al., 2018), Nevertheless, higher than the results in Sudan- 1.45 (Mustafa et al., 2017). The variability in the MOI is indicative of the different levels of malaria transmission in *P. falciparum* strains between the study districts involved in the current study. Also, Substantial human mobility among the districts may be the reason behind the statically similar malaria transmission observed among the districts. On the other hand, human host immune selection may also have maintained the antigenic diversity and selection of clones (Bushman et al., 2018).

The mean COI ranged from 1.44 to 2.07 among the age groups. The highest mean COI is within the 15-19 years age group representing 2.07 (95%CI: 1.79-2.36). The lowest mean COI is 1.44 (95%CI: 0.75-1.73) and among the 10-14 years age group with a standard deviation of 0.75. The COI was also found to be high in females both in frequency and proportions distribution but this may be due to a large number of female participants in the current study. Thus, the high mean MOI among the age group 15-19 years and the female gender can be ascribed to high exposure to mosquitoes as a result of cultural-related activities in the study area and the untamed lifestyle of the teenagers. Females are involved in hawking and trading along the road networks deep into the night to serve travellers passing through the region to neighbouring Ashanti, Western, and Greater Accra regions. The high MOI

found in this category of participants indicates high malaria transmission and may be due to the boosted parasite-specific immunity resulting from high exposure as observed in residents of high malaria transmission regions in Kenya (Touray, 2020). However, among this age group and gender, there may also be an increase in the number of strains harboring resistance-conferring mutations (*Pfmdr-1*, *Pfdhfr*, and *Pfdhps*) as observed in the current study and therefore are of the ability to evade the host immune response.

In the current study, the coastal zone recorded a mean clone of 1.68, whilst the forest zone recorded 1.86. No statistical significance was observed for the comparison of mean MOI in coastal and forest zones (*P-value* = 0.0681) despite the high prevalence of polyclonal infections among isolates from the coastal study sites, compared to that of the forest sites. In a recent study in Ghana, the mean MOI in the forest and coastal zones were similar (mean MOI = 1.4), whilst the genetic diversity in the Sahel and Forest zones was higher than diversity in the Coastal zone during the rainy season (Amoah et al., 2021). A similar report indicates that the diversity in the Msp2 locus in Obom and Asutuare was not significant even though the more polyclonal infection was observed in Obom (high malaria transmission intensity) than in Asutuare (low malaria transmission intensity) (Abukari et al., 2019). Also, parasites isolated in the lowland coastal region of Kenya presented with the lowest genetic diversity when compared with parasites from the highlands (Ingasia, Cheruiyot, Okoth, Andagalu, & Kamau, 2016). Varying malaria transmission intensity within the zones may account for observed discrepancies.

The current study recorded a high genetic diversity in the dry season with a mean clone of 1.81 whilst the rainy season recorded 1.79, however, this finding was not statistically significant (*P-value* = 0.8034). The findings contradicts with previous findings that the dry season is associated with reduced MOI (Ayanful-Torgby et al., 2018) whilst a similar study in southern Ghana also reports low MOI during the peak season in Obom (Adjah et al., 2018). However, there were disparities in malaria incidence during the rainy season being the highest in Konongo (24.1%) and lowest in Ada (12.8%) compared to the dry season with high prevalence in Tamale (13.6%) and lowest in Kumbungu (7.1%) (Amoah et al., 2021). In Cote d'Ivoire, genetic diversity among asymptomatic and symptomatic malaria-infected children in three epidemiological areas showed that the diversity in the *Pfmsp*1 allele family is higher during the rainy season (Gnagne et al., 2019). High MOI in the dry season may imply that genetic diversity aids in the adaptability of the parasites to existing interventions, increasing parasite survival and successful transmission in the dry season. These variations also iterate the significant role of micro-ecological factors in parasite diversity and transmission in the study area and beyond (Amoah et al., 2021). Also, another difference in parasites' genetic diversity between the ecological zones may be because resistant parasites persist longer in a low-transmission setting than in a high transmission as reported in a study on within-host competition (Bushman et al., 2018). Comparisons of genetic diversity between the current study and other studies may be limited as different loci and methods were used to calculate MOI. However, the lack of a significant association between COI and *P. falciparum* prevalence in the seasonal analysis compared to other

studies could be due to the high stable malaria transmission across the study districts.

Malaria outcomes may improve significantly if detected early and treated with effective antimalarials (Baghbanzadeh et al., 2020). Also, the identification of molecular markers has proven to be an effective tool for the monitoring and tracking of parasite genetic diversity and its impact on antimalarials, providing valuable information for assessing resistance trends and confirming resistance (Okell et al., 2018). Monitoring of antimalarial resistance was previously based on therapeutic efficacy studies (TES), which lengthened the time frame in identifying molecular markers of resistance to Chloroquine (CQ). Presently, the detection of artemisinin resistance (*Pfkelch13)* was faster due to readily available molecular technologies (Ndwiga et al., 2021). With the aid of Selective Whole Genome Amplification (sWGA), this study examined the drug-resistance genetic markers of *P. falciparum*, which was of high prevalence in the current study and documented as the deadliest malarial parasite in sub-Saharan Africa.

Chloroquine (CQ) was the most affordable yet effective antimalarial drug for decades (Ndwiga et al., 2021) until the target parasites became resistant. CQ resistance (CQR) was attributed to the *Pfcrt* 76**T** mutation in the *P. falciparum* Chloroquine resistance transporter (*Pfcrt*) gene (Djimdé et al., 2001; Fidock et al., 2000), which finally led to the retraction of the drug from clinical use (Hemming-Schroeder et al., 2021). More recently changes in this marker have highlighted a reversion to CQ sensitive (CQS) (Wamae et al., 2019).

In the current study, amino acids at codons 74, 75, and 76 were found to be mutated in the *Pfcrt* gene, similar to findings from *P. falciparum* imported to Shandong province, China (Xu et al., 2018), in parasites in Chinese patients infected in Africa with *P. falciparum* (Zhao et al., 2021), and in the Greater Accra region of Ghana, at a frequency 11.6% (Aninagyei et al., 2020). This resulted in a high prevalence of CQ-sensitive parasites (97%) followed by CVM/**I**N/**E** K/**T-2.5%** (multiple clonal infections) and CQ-resistant parasites (0.5%). Similar studies in the Central region indicated a low prevalence of the CQR alleles recording 142 out of 184 (77.17%) for the CQ-sensitive marker K76 (Asare et al., 2021). Such high dominance of the *Pfcrt* wild-type haplotype has also been reported at 80% in Guangxi Shanglin Hospital (Zhao et al., 2021), 97.3% in Katete district in Zambia (Mulenga et al., 2021), 100% in Mozambique (Chidimatembue et al., 2021), 84.7% in Chad (Das et al., 2022), and 100%, 88.5% and 62.29% in Cameroon (Fontecha et al., 2021; Tuedom et al., 2021) as well as current reviews on Chloroquine resistance profile among SSA countries after the drug was withdrawn from regular use (Njiro et al., 2022; Ocan et al., 2019). In other jurisdictions, the prevalence of the CQR allele (76T) is still very palpable, such as in the China-Myanmar border area, where *Pfcrt* CQ-resistant haplotypes (CVIET or SVMNT) were observed in 100%, 98%, and 36.6% of the parasites (He et al., 2019), in Nigeria - CVIET (61.11%) and CVMNK (33.33%) (Adam et al., 2021) and Angola with CQR haplotypes at a prevalence of 73% (Ebel et al., 2021). The disparities in prevalence in the CQ-resistance maker k76T can be attributed to the removal of Chloroquine from clinical care which resulted in a decrease in the drug selection pressure (Njiro

et al., 2022; Ocan et al., 2019). However, the high prevalence of CQ-resistant haplotype CVIET in other jurisdictions may be due to the co-existence of *P. falciparum* and *P. vivax* infections. This is because CQ remains a standard regimen for *P. vivax* malaria treatment and a source of continuous drug pressure in *P. falciparum* particularly on multiple infections (Fontecha et al., 2021). The SVMNT haplotype associated with AQ resistance in Tanzania and Angola (Alifrangis et al., 2006; Gama et al., 2010) was not detected in the present study as well as in a study in the DRC (Yobi et al., 2021), even though AQ is one of the partner drugs of ACTs used in many endemic countries including Ghana.

There were varying results in the distribution of CVNMK haplotype among the districts, age groups, gender, ecological zone, and season. However, the odds of acquiring a CVIET haplotype showed that there are higher odds among the male gender, coastal ecology, dry seasons, and district of residence but not statistically significant. Also, the odds were highest and statistically significant among the age group 10-14 years. Comparable another study in the central region reported that *Pfcrt* K76 was most prevalent in the age group 16–30 years at the Elmina study Site (Asare et al., 2021) . However, In the DRC, the highest prevalence was in the age group 6-12 (29.3%) and the least in the age group 0-5 years (23.2%) (Yobi et al., 2022). The genetic similarities and differences between forest and coastal parasite populations may be due to parasite introduction by human travel between the two ecological zones influencing parasite natural selection and genetic drift (Ingasia et al., 2016). Also, the districts in the Central region, are well connected by a vibrant road network, which further aggravates the transfer of

genes during commercial, educational, recreational, and cultural heritage activities within and across regions. In addition to human travel, the population structure of the malaria parasite may be affected by parasite genetic makeup, site-specific epidemiology, and malaria control measures in the district.

Characterization of pyrimethamine drug-resistance parasites is based on the number of mutations present (single, double, triple, and quadruple mutants) (Chaturvedi et al., 2021). Only five percent (5%) of successfully sequenced *Pfdhfr* gene was of the wild type in the current study. A low prevalence (n=9, 6.5%) of the wild-type haplotype has also been recorded in Sudan (Hussien et al., 2020) and Ghana 6.5% (Tornyigah et al., 2020). In this study, mutations were observed in all studied codons of the *Pfdhfr* gene (N51I, C59R, S108N, and I164L), with a mutation at codon 164 observed at a very low frequency. The 164L mutation has been implicated in resistance to Cycloguanil, the active form of proguanil (Quan et al., 2020). Similar studies have also reported the mutation I164L at a different frequency: 2% in Angola (Kaingona-Daniel et al., 2016), in 112 isolates from 2004 to 2006, and from 2009 to 2012 in Indonesia (Basuki et al., 2018), 58% in Thailand (Kuesap et al., 2022) and up to 80% in Uganda (Asua et al., 2021). However, the mutation was not recorded in Nigeria (Quan et al., 2020) in Cameroon (Tuedom et al., 2021). In Ghana, the I164L mutation was previously reported at a frequency of 0.9%, 1/112 (Mama et al., 2022) and at 12.3% among Ghanaian migrant workers returning to Guangxi from Ghana (Zhao et al., 2020). Nevertheless, the mutation was not recorded in Kpone and Mamobi in the Greater Accra region(Tornyigah et al., 2020).

Results from the current study revealed that the prevalence of isolates carrying the *Pfdhfr* IRN triple mutation (76.6%, 275/359) is high in the study sites. Similar results have been reported in studies from different parts of Ghana such as Kpone and Maamobi -79.7% (Tornyigah et al., 2020), and 71.4%, 70.3%, and 71.1% among ANC attendees, placenta blood and OPD attendees (Afutu et al., 2021). In other parts of the African continent, similar observations were made. In Angola, approximately 97% of isolate carried the triple mutation (Ebel et al., 2021). Similarly, 763/772 (98.83%) and 674/724 (93.09%) *Plasmodium* falciparum isolates in Mfou and Tibati, respectively were found to harbour this mutation in the Democratic Republic of Congo (DRC) (Tuedom et al., 2021). Additionally, a prevalence of 68.8% was recorded in Zambia (Chaponda et al., 2021), 89.8% in Benin(Svigel et al., 2021), 93.8% in Nigeria (Quan et al., 2020) and 78% in Kenya (Osoti et al., 2022) and 84.8% in Chad (Das et al., 2022).

The high prevalence of N51I, C59R, and S108N in the *Pfdhfr* gene confirms reports of this mutation being the most prevalent in Asia and Africa (Chaturvedi et al., 2021; Kuesap et al., 2022), and is associated with high pyrimethamine resistance in West Africa (Balogun, Sandabe, Sodipo, Okon, & Akanmu, 2021). However, in other jurisdictions, the most prevalent amino acid change observed in pyrimethamine resistance was the double mutant N51I-S108N (70%) and the triple mutant, IRN (30%) (Warsame et al., 2017).

The triple mutant IRNI was recorded in very high frequencies (> 50%) in all age groups but significantly high in the age group 10-14 years (n=20, 83.8%), in the rainy season (n=190, 81.2%), and among female participants, 165/212 (77.8%) than in males 110/147 (74.8%) comparable to findings in

female participants in Nigeria (Balogun et al., 2021). The quadruple mutant haplotype IRN[I/L] was recorded among the age group 15-19 years (2, 5.1%), and found unique to the rainy season and the female gender (2, 0.9%) among participants.

Logistic Regression of factors influencing putative drug resistant haplotype to *Pfdhfr* gene showed high odds among age groups 5-9yrs, 10-14yrs, and 15-19yrs. Similarly, high odds were recorded among males compared to females. The odds were however low in all study districts as well as in the coastal zone compared to the forest zone. The odds among the age group, gender, ecological zone, and study districts were not statistically significant. However, even though the odds were low in the dry season compared to the rainy season, this showed statistical significance (*P-value*-0.036). Varying prevalence among the age groups, gender, ecological zones, and seasons may be due to variations in patterns of malaria transmission and the prevalence of pregnant women and children who are the main beneficiaries of pyrimethamine antimalarial in the districts. This population-based prevalence may also affect the drug selection on resistance alleles due to the high consumption rate. Thirdly, the varying health-seeking behavior of the gender and within the seasons may equally have the potential to reduce or inflate estimates of mutant allele frequencies.

In the *Pfdhps* gene, the wild-type SAKAA haplotype was nearly non-existent with a prevalence of 2/314 (0.6%). This finding compares with recent reports from Chad with 5 out of 348 isolates (1.5%) harbouring the wild-type allele (Das et al., 2022), whilst contrasting the 39.62% recorded among returning Chinese migrants from Africa (Yan et al., 2021). However, studies

in neighbouring greater Accra region did not record the wild-type alleles (Aninagyei et al., 2020; Tornyigah et al., 2020). The single point mutation S436A/F and A437G which were the most common in this study is also common in west Bengal at a Prevalence of 30% (Chatterjee et al., 2017).

The single mutant haplotype SGKAA (32.8%) prevailed amongst the single point mutations recorded as well as among the polyclonal samples. These findings corroborate with findings in Ghana (13, 16.4%) (Aninagyei et al., 2020), 94.80% in Mfou, and 86.36% in Tibati in the DRC(Tuedom et al., 2021), 95.8% in Nigeria (Quan et al., 2020) and at 40.6% in Angola (Ebel et al., 2021). In contrast, the SGKAA haplotype was of a low prevalence among haplotypes in a cross-sectional survey in Chinese migrants returning from Africa (Yan et al., 2021), and in Ghana, Tornyigah recorded a higher prevalence of double mutation at codons 436 and 437 (**A/FG**KAA) in 71.6% (101/141) isolates (Tornyigah et al., 2020). The distribution of SGKAA single mutant was approximately equal among the gender -females (33.2%) and males (33.1%) but was proportionally high in the coastal zones (35%) compared to the forest zone (32.2%). This haplotype was recorded at a high proportion in the dry season (36.3%) than in the rainy season (31.3%).

The double mutants SGEAA was of a low prevalence as a monoclonal isolate (1, 0.3%) and among the polyclonal (n-12) samples ([S/A]G[K/E]AA (5,1.6%) [S/A][G/A][K/E]AA (3, 0.9%), SG[K/E]AA (2,0.6%), AG[K/E]AA (1,0.3%) and S[G/A][K/E]AA(1,0.3%). The SGEAA haplotype was recorded at a prevalence of 0.7% (Tornyigah et al., 2020) and approximately 18% in the greater Accra region (Aninagyei et al., 2020), Similarly, low prevalence of the haplotype, was recorded at a prevalence of, 0.12% in Mfou and 0.53% in

Tibati in Cameroon (Tuedom et al., 2021), and 1.2% (2/167) in Nigeria (Quan et al., 2020). Additionally, a moderate prevalence of 32.6% was recorded in DRC (Kayiba et al., 2021), 37.5% in Angola (Ebel et al., 2021) and 41% in Sudan (Hussien et al., 2020). The current study however, did not record the K540T and K540N previously recorded in Indonesia (Basuki et al., 2018) and in India (Das et al., 2016) respectively. The SGEAA haplotype was recorded from a female sample probably due to their large number in the current study.

Very few isolates from the current study had mutations at codons A581G and A613S. However, the A581G and A613S/T mutations were found predominantly in the absence of the K540E mutation vital to attain a super resistant status. Comparably, a low prevalence of these mutations was also observed in other studies in Benin where K540E, A581G, and A613S were recorded at frequencies of 2.6%, 2.6%, and 4.6% respectively (Svigel et al., 2021). A low frequency of mutations at codon 581G has also been described at a frequency of 2 out of 322 samples sequenced (Osoti et al., 2022), in 1(3.1%) in Angola (Ebel et al., 2021) and at 1.1% in Tanzania in 2019 (Bwire et al., 2020). Contrary to this finding, is the high occurrence of mutations at codons A581**G** and A613**S** observed at a prevalence of 31.1% (52/167) and 41.9% (70/167) in Nigeria (Quan et al., 2020), and up to 67% in Uganda (Asua et al., 2021). However, the prevalence in the current study is below the WHO thresholds to warrant any change in the use of IPTp or to speculate that SP may negatively influence the birth weight of infants (> 95% for K540**E** and > 10 % for A581**G**) (WHO, 2020).Even though this current study was prospective, it did not take into account birth outcomes to correlate this result in the study locations.

This study did not detect SGEGA, a "super resistant" haplotype, common to the other study population as reported at 26.2% in Sudan(Hussien et al., 2020), in 2 isolates in Tibati, Cameroon (Tuedom et al., 2021) and in parasites in Asia (Chaturvedi et al., 2021).

Logistic Regression of factors influencing the Putative Drug Resistant Haplotype to the *Pfdhps* gene showed high odds among age group 5-9yrs, and 15-19yrs and equal in age group 10-14ys as well as age group 60yrs plus. Similarly, high odds were recorded in districts Abura-Asebu Kwamankese, Cape Coast Metropolis, and Gomoa East District. The odds of acquiring a drug-resistant haplotype were twice as much in the coastal than in the forest zones. The odds were however low among males compared to females and similarly in the dry season compared to the rainy season. The odds among the studied variables not statistically significant.

Sulphadoxine-Pyrimethamine (SP) has been a preferred, affordable alternative since CQR. However, treatment failure coupled with observed molecular markers of resistance in the *P. falciparum* dihydrofolate reductase (*Pf*dhfr) triple mutation -IRN and *P. falciparum* dihydropteroate synthase (*Pf*dhps) double mutants-KE rendered it ineffective in radical cure and hindered its extensive use, prompting the change in treatment policy to ACTs (Ndwiga et al., 2021; Roux et al., 2021). However, differences in the *Pfdhfr* and *Pfdhps* genotypes have been observed across Africa (Svigel et al., 2021), where SP remains the backbone of intermittent preventive treatment in pregnancy (IPTp/ IPT in infants) and seasonal treatment for children (SMC) within endemic areas in the Sahel region (Afutu et al., 2021; Mama et al., 2022). In addition to the most common alleles, the recent emergence of "super

resistant" alleles, such as *Pfdhfr* 164L, *Pfdhps* 581G, and *Pfdhps*, 613S/T may further diminish the effectiveness SP (Hemming-Schroeder et al., 2021; Svigel et al., 2021).

The quadruple mutants (N51**I**, C59**R**, S108**N** plus A437**G**) were the most widespread in the study sites and compares with studies in Congo where 19 out of 43 isolate genotypes (44.2%) were of the quadruple mutation among pregnant women (Kayiba et al., 2021), 40.6% (Ebel et al., 2021) and 93.1% (108/116) (Boukoumba et al., 2021), 90% (Tuedom et al., 2021). Similar records emanated from other parts of Ghana: 72.6% and 68.0% in Kintampo and Navrongo respectively between 2012-2013 as well as 81.1% and 81.7% in the same localities in 2016-2017 (Abugri et al., 2018), 11.9%, 11.1% and 13.3% in ANC attendees, placenta blood and OPD attendees (Afutu et al., 2021).

A low prevalence of the Quintuple mutants consisting of *Pfdhfr* 51I/59R/108N and *Pfdhps* 437G/540E considered to be fully resistant to SP were observed in this study and also at 0.5% among OPD attendees in Sekondi–Takoradi (Afutu et al., 2021). However, in Kenya, quintuple mutants were present in 91.3% of the isolates collected in 2015(Hemming-Schroeder et al., 2021), 37.5% (Ebel et al., 2021), and 57.8% (Boukoumba et al., 2021). In other areas, observation of mutations at codon A437G, K540E, A581G, and A613S have cumulatively resulted in septuple haplotypes (e.g., IRNL-GEG made of *Pfdhfr* N51I, C59R, S108N, and I164L with *Pfdhps* A437G, K540E, and A581G), and even octuple mutant haplotype (e.g., IRNI-VAGKGS carrying *Pfdhfr* N51I, C59R, and S108N, along with *Pfdhps* I431V, S436A, A437G, A581G, and A613S) in the Congo (Nkoli Mandoko et al., 2018). In

Zambia, 68.8% and 9.4% expressed the quintuple mutant and sextuple mutant respectively (Chaponda et al., 2021). Also, no isolate of the current study carried mutations in all codons as seen in *Pfdhps* codons studied in the Chinese immigrant (I431V, S436A, A437G, K540E, A581G, and A613S) (Yan et al., 2021). However, in Pakistan, SP-quadruple and SP- quintuple mutants were not observed (Yaqoob et al., 2018).

Logistic Regression of factors influencing putative drug resistance haplotypes to SP among participants in the study sites indicates an almost equal odd among all the age groups. The odds were however low in males and in the coastal zones when compared to the female gender and forest zones respectively. The odds were equally low among all the districts except Abura - Asebu Kwamankese. However, none of these variables showed statistical significance.

The emergence of these mutations in the study population suggests the indigenous emergence of the mutant allele in the districts and probably points to a transitory stage toward the loss of the efficacy of SP. These mutations in the study area may be attributable to the selective pressure from the prior use of SP as a monotherapy, and current use of the CQ-SP combination as first-line treatment for more than two decades as well as prophylaxis IPTp with SP for malaria in pregnancy as recommended by the WHO.

The identification of the validated drug-resistant alleles in the *Pfkelch13* genes in SEA (Aninagyei et al., 2020; Ndwiga et al., 2021; Uwimana, 2020), coupled with reports that in some regions of Africa and South America, malaria parasites respond poorly to ACT. This has raised the possibility that high burden high impact (HBHI) might be in danger due to the

de novo development of artemisinin resistance or the importation of drug resistant parasites.

Countries may be at risk for artemisinin resistance due to *de novo* emergence or the importation of a resistant parasite (Maniga et al., 2021; Nzoumbou-Boko et al., 2020; Zhao et al., 2020; Zupko et al., 2022). Markers observed to propagate ACT resistance in the South East Asia include C580Y, R539T, Y493H, I543T, F446L, P553L, N458Y, P574L, and R561H. Whilst other markers have been categorised as candidate markers for artemisinin partial resistance which include P441L, G449A, C469F/Y, A481V, R515K, P527H, N537I/D, G538V, V568G, R622I AND A675V (WHO, 2020; Matrevi et al., 2022). Irrespective of these findings, parasites lacking *Pfkelch 13* mutations but showing ACT-resistant phenotypes were identified in Cambodia (Mukherjee et al., 2017).

With the use of ACT in Ghana since 2006 and 2009 respectively, there is a growing concern that drug-resistant parasites are evolving and are under selective pressure. Yet, very little is known about the circulating polymorphisms to ACTs in Ghana and the Central Region. Thus, this study also presents findings on the occurrence of point mutations in the *Pfkelch 13* gene from clinical isolates. The current study revealed that the prevalence of wild-type *Pfkelch 13* genes (52%) was slightly higher than that of mutated genes (48%). Of the mutated genes, single (28), double (15), triple (2), and quadruple (1) mutations were recorded. Similarly, a recent study in Ghana recorded mutations ranging from one SNP per codon to three SNPs per codon in a single isolate (Matrevi et al., 2022). The prevalence of mutant alleles was higher compared to the 3.9% in 2015 to 19.8% in 2019, recorded in Northern

Uganda (Balikagala et al., 2021), at a 3.8% mutation rate from 2012 to 2017 in *P. falciparum* isolates imported from Angola into the Henan Province, China. Reports (Zhou et al., 2019), 2.5% in 2014 and 4.5% in 2015 in Rwanda (Bergmann et al., 2021), and in a recent study in Ghana, 22% (214/977) had *Pfkelch k13* mutations (Matrevi et al., 2022). However, a high prevalence of wild-type alleles has been recorded in Cameroon (169/175, 96.6%) (Eboumbou Moukoko et al., 2019). In other studies, the validated *Pfkelch 13* resistance mutation C580Y detected in the Central Region (Mensah et al., 2020) and the Greater Accra Regions (Aninagyei et al., 2020) of Ghana were not observed in the current study.The high occurrence of *Pfkelch 13* mutations may be a result of selective pressure exerted in managing the high malaria prevalence in the Region (Ejigu & Wencheko, 2021), and the abuse of antimalarial medications, which results in the development of drug resistance as postulated earlier (Maniga et al., 2021; Matrevi et al., 2019; Matrevi et al., 2022).

The prevalence of the mutant genes varied across the district with the highest prevalence among the single mutants in Abura –Asebu- Kwamankese (44.2%) and the least recorded in Agona Swedru Municipality (33.3%), however, the highest prevalence in polyclonal haplotypes was recorded in Gomoa East District (23.9%) and the least in Assin Central Municipality (5.6%). Disparities within districts may be due to the impact of the diverse ecological niches in each site on the parasite's genetic structure. Most of the SNPs recorded were in low frequencies (below 5% prevalence) and agrees with reports that Africa has low frequency of non-synonymous mutations

(Asua et al., 2021; Behrens et al., 2022; Kamau et al., 2015; Moser et al., 2021; Ndwiga et al., 2021; Ocan et al., 2019).

Of the six validated resistance-conferring *Pfkelch 13 mutations* (M476I, P553L, R561H, P574L, C580Y, and A675V) in African countries at moderate to the low frequency of 4.1% or less (Aninagyei et al., 2020; Imwong et al., 2017; Uwimana, 2020), only the M476I mutation was observed in the current study. Two previously detected *Pfkelch 13* propeller mutant alleles, N537D and V568A correlated with decreased parasite clearance or ring survival (the RSA0–3 h phenotype) in Southeast Asia (Ménard et al., 2016) and Kenya (de Laurent et al., 2018), were also detected in the current study. The occurrence of these markers contradicts previous genomic epidemiological research, which indicated that *P. falciparum* parasites with verified artemisinin resistance mutations were not present in Southeast Asia (Maniga et al., 2021), in Pakistan (Yaqoob et al., 2018), Burkina Faso (Zupko et al., 2022) and some parts of Nigeria (Ajogbasile et al., 2022).

This research found alterations that have previously been reported in African malaria parasites and are most probable candidates for treatment resistance indicators. Among these is the A578S, along with its variant mutant A578V. However, variant A578V was recorded in higher allele frequencies than the A578S in the current study. The mutant is equally not new to Ghana as it has been previously recorded in other parts of the country (Matrevi et al., 2019), in greater Accra region (Aninagyei et al., 2020) and the Central Region (Mensah et al., 2020). The A578S allele is the most prevalent *Pfkelch 13* SNP observed in Africa and has been reported in Angola (Rodrigues et al., 2022), and the Democratic Republic of the Congo (Mayengue et al., 2018). It was

part of the three SNPs -A578S, G592V, and V637I noted in a study in 2015 (Kamau et al., 2015) as well as those (F446I, A578S, and K189T) found in India (N. Mishra et al., 2016) and Rwanda (Uwimana, 2020). It was also detected in post-ACT parasites in two different study sites, Kombewa (at a frequency of 4.3%) and Kisii (at a frequency of 2.1%) in Kenya (de Laurent et al., 2018). The functional impact of A578S is unclear, however, recent studies indicate substitution Ala578Ser, in the *Pfkelch 13*-propeller domain has not been confirmed to mediate delayed clearance of parasites in both clinical and in vitro studies (de Laurent et al., 2018; Maniga et al., 2021) and the A578S SNP may cause the propeller domain's functionality to be disrupted, according to computational modelling and mutational sensitivity predictions (Voumbo-Matoumona et al., 2018). Other alternative forms A578P (Wa) and A578A (Sunyani) recorded in the Upper west and Brong region were not found in this study (Matrevi et al., 2019). Another study in Senegal recorded the alternative form, A578D which was not observed in the current study (Gaye et al., 2020).

This study also recorded A676D mutation shown to induce significantly higher rings stage survival rates than the wild-type parasites in the China-Myanmar border area (Zhang et al.2019) but it does not seem to confer ART resistance (He et al., 2019). The A676D SNP has already been discovered in several investigations from the border between China and Myanmar (He et al., 2019).

Also, M579T a variant of the mutant M579I, previously identified in Equatorial Guinea and linked to an increased parasite clearance time on day 3 following dihydroartemisinin-piperaquine (DHA-PIP) treatment (Lu et al.,

2017) was also recorded in the current study. The M579T has been reported in India (Mishra et al., 2017), and Ghana (Matrevi et al., 2022).

The remaining S485G, I405V, I406T, I684V, K420R, M460I, M460V, E426K, F614S, and G591V amino acid changes, are unique mutations, and in vitro research is required to understand how they relate to artemisinin resistance.The findings concerning the *Pfkelch* 13 gene, corroborate with previous research that found SSA parasites to harbor a variety of uncommon and unusual Pfkelch 13 mutations(de Laurent et al., 2018; Ikegbunam et al., 2022; Ménard et al., 2016; Taylor et al., 2015). Nonetheless, the diversity in mutations observed in the current investigation and other studies could be a result possibly as a consequence of the adaptation due to the different levels of selective pressures to the Artemisinin combinations currently in use in the region due to high use or abuse of ACT as more than 95.8% of the total prescription of antimalarials are artemisinin-based combinations (Ikegbunam et al., 2022). Also, findings from this study show that a given endemic setting may favour variant forms of *Pfkelch 13* mutants.

Of the 5 districts studied, SNPs were recorded in all but of high prevalence in the coastal zones (38.7%) compared to the forest zones (33.8%). Even though there was an almost equal proportion of multiple mutations, they were of high frequency in the coastal zone compared to the forest. Despite the vast diversity of the *Pfkelch* 13 gene in this study, some SNPs were found unique to the forest and coastal zones respectively. SNPs unique to the forest zone were S485G, A621K, E426K, F614S, G591V, I684V, K438R, M460I, M579T, V520A, V568A and V603A whilst SNPs unique to the coastal zones were E612K, I405V, I406T, K420R, L429S, M460V, R513H, V534A and

W518L among the single SNPs. All multiple (triple and quadruple) mutations identified in the study were unique to the forest zones within the study area. However, among the double mutations, A504D D501G, A621D M476I, E651G V356A, N609S I551T and W660C P413L were common to the forest zone whilst W660S P413L, F506L P413L, A578V N537D and A504D P413L were unique to the coastal zone. This finding agrees with reports that individual *Pfkelch 13* nonsynonymous mutations have shown restricted geographic localization (Ménard et al., 2016). Other research in Ghana have also discovered unique locus mutations at different sites and ecological zones, and it has been observed that the coastline region, which includes Accra and Cape Coast, has more novel SNPs than the forest. The unique SNPs peculiar to the ecological zones were, C580R and K669E/N for coastal, and the Q613P (2 samples) found in Cape Coast 2019, M579T/Y and D584L for forest and N554P and A569P for the savannah (Matrevi et al., 2022). None of the SNPs unique to the coastal zone in Matrevi's study were recorded in the current study but both studies recorded the M579T SNP in the forest zone as well as variant mutants at codon 534 in the coast (V534A recorded in the current study V534V found in Cape Coast and Sunyani in Matrevi's study in 2019 (Matrevi et al., 2019).

There was diversity in the SNPs even between locations in the same ecological zone whereas other SNPs were unique to each district but observed at different frequencies. For instance, the quadruple mutant, A676D A504D D501G P413L was observed only in GED, whilst the triple mutants A578V P413L R393I and A676D A578V P413L were observed in ACM and ASM

respectively. Also, the double mutation W660C P413L and W660S P413L were observed in GED and AAK respectively.

In other studies, the validated *Pfkelch 13* resistance mutation C580Y detected in the Central (Mensah et al., 2020) and the Greater Accra Regions (Aninagyei et al., 2020) of Ghana were not observed in the current study. A high prevalence of *Pfkelch* 13 mutations was found in the age group 60plus (43%) and closely followed by 5-9yrs (40.3%), whilst multiple mutations were most prevalent in the age group 10-14yrs (19%) and closely followed by age group 20-59yrs (18.7%). The frequency of multiple and single mutants in the *Pfkelch 13* was proportionately greater in men than in females. The proportion of both single and multiple mutations was greater in the coastal zone than it was in the forest zone. The proportion of single *Pfkelch 13* was equally distributed in the seasons, however, the rainy season recorded a higher proportion of the multiple mutations and may result from higher transmission intensity during the rainy season as recorded in the study.

Logistic Regression of factors influencing *Pfkelch13* resistance in *Pfkelch13* among participants showed low odds in age groups below five, 5-9yrs, and 10-14yrs but higher odds in age group 60plus followed by age group 15-19yrs. Nevertheless, the odds were statistically significant among the age group below 5yrs only. Low odds were recorded among the male gender and dry season, whilst the coastal zone showed higher odds when compared to the forest zone. However, the odds recorded among the gender, ecological zones, and seasons were not statistically significant. Among the districts, however, high odds compared to the reference was recorded in all district but only comparisons with Abura Asebu Kwamankese district and Gomoa East district

showed statistical significance. These geographical differences in *Pfkelch 13* mutations may also be a result of the variations in the parasites' genetic origins and pharmacological histories. The novel mutations in each ecological zone may result from the recombination of genetic material and spontaneous evolution of the gene, whilst the genes common to the ecological zones may be a result of gene flow among the locations in each zone.

Since 2009, Ghana has employed Artemether Lumefantrine (AL) and Artesunate Amodiaquine (ASAQ) as its two primary antimalarial regimens, with AL being the more popular option and ASAQ serving as a fallback in cases when AL is unavailable or impractical. The sustenance of the efficacy of these partner drugs is by the *Pfmdr1* gene. Multiple antimalarial medications have been shown to be resistant due to mutations in the *Pfmdr1* gene at codons 86, 184, 1034, 1042, and 1246. The N86, Y184F, and the D1246 SNP are connected to a reduction in sensitivity to Lumefantrine (Chidimatembue et al., 2021) whilst *Pfmdr1* YYY (86Y, Y184, and 1246Y) was selected after being exposed to artesunate and amodiaquine (ASAQ) (Mensah et al., 2020).

The wild-type NYD haplotype was present in the current investigation but at a low frequency of 22.3%, whilst the higher prevalence of 55.3% and 84.11% were found in Cabinda, Angola(Ebel et al., 2021) and includes portions of Ghana's Central region (Asare et al., 2021). Also, the Y184F mutant alleles were the most prevalent mutation resulting in a high prevalence of the observed haplotypes, N**F**D (44%), N(Y/F)D (23.5%) and NYD (22.3%). Similar to the prevalence of *Pfmdr1* 184F (> 60%) in a study in parts of the central region (Mensah et al., 2020). The NFD haplotype was however of a lower frequency (5.4%) in the greater Accra region of Ghana (Aninagyei et

al., 2020). A high prevalence (61.5%) of the NFD haplotype was also detected in pre-treatment samples and 100% of post-treatment samples from research in Mozambique (Chidimatembue et al., 2021) and over 50% prevalence (172/331) in Chad (Das et al., 2022) and 36.8% in Angola (Ebel et al., 2021). A research in Wuhan revealed similar results, recording a low prevalence of N86**Y** and a high prevalence of Y184**F**, with 4.72% and 47.17% mutations, respectively (Cheng et al., 2021) . In another study, the wild-type haplotype was recorded at 31.2% prevalence and NFD at 48.7%(Zhao et al., 2021), while in a research conducted in Cambodia, all mutant Pfmdr1 haplotypes were N86/184F/D1246 (Mairet-Khedim et al., 2021). Additionally, in other research, parasites with the NFD haplotypes tolerated greater (15-fold higher) Lumefantrine blood concentrations than parasites with the 86Y-184Y-1246Y haplotype (Ontoua et al., 2021).

Additionally, the findings of the present investigation indicates a low incidence of Pfmdr1 D1246 mutant alleles, a mutation linked to CQ, ASAQ, and quinine resistance. Similarly, a low incidence was recorded in another study in the Central region at a prevalence of 2/76 (2%), 3/97(3%), 1/76(1%), and 0/60(0%) in 2014, 2015, 2016, and 2017 respectively(Mensah et al., 2020), and in two different localities in Mfou (1.45%) and Tibati (5.97%) in Cameroon (Tuedom et al., 2021). Prevalence of the 1246Y mutant allele was recorded at a range of 1.3% to 6.7% among the districts in Gabon (Maghendji-Nzondo et al., 2016), while a prevalence of 5.26% was found in Yobe state, Nigeria (Adamu et al., 2020) and 7.7% in Uganda (Achol et al., 2019).

The current study observed isolates with the triple mutant, YFN (N86Y/Y184F/D1246N) at a prevalence of 33 (9.8%). This haplotype is not

unique as it has been previously observed at an almost equal proportion in the Greater Accra region (Aninagyei et al., 2020). In the present research, none of the isolates had the YYY haplotype associated with amodiaquine resistance in Africa, however, it was recorded at a low prevalence (2%) in a previous study (Mensah et al., 2020) and 2.6% in Angola (Ebel et al., 2021). This haplotype was also not recorded in recent reports from Chad (Das et al., 2022) . The variations in treatment protocols and selective pressure shown among the nations may be responsible for the observed variations in haplotype prevalence among them (Zhao et al., 2021). However, the high frequency of NFD haplotype may be because of easy access to the Artemether Lumefantrine medications over the counter from pharmacies as observed in the Democratic Republic of Congo (Yobi et al., 2021). The results confirm that AL is used extensively among the general population as suggested by the results from East–Central Gabon (Ontoua et al., 2021), Kenya (Hemming-Schroeder et al., 2018), and Asia (Menard & Dondorp, 2017), which associated a significant selection of NFD and suggests a potential early indicator of diminished AL effectiveness in the future.

The odds of acquiring a mutant *Pfmdr*1 haplotype were found higher in males probably because of previous AL-treatment (self-medication) compared to females (Quaresima et al., 2021; Russo et al., 2017) and in the coast compared to the forest zones. It was also highest in Gomoa East District (GED) and least in Agona Swedru Municipality (ASM). However, none of these variables were found statically significant. Meanwhile, even though the odds were less in the dry season compared to the rainy season and statistically contradicting the hypothesis that, in the absence of persistent pharmacological

pressure throughout the dry season, these alterations may lose their competitive edge (Ehrlich et al., 2021). This is supported by the correlation between getting malaria in the dry season compared with the rainy season as reported in the current study. The results revealed the effect of ecological niches created as a result of climatic changes on the effect of parasite sustenance.

In addition, commonly available ACTs such as artesunate–amodiaquine and Artemether Lumefantrine, Dihydroartemisinin–Piperaquine pose different selective pressure in sustaining parasite drug resistance. The exonucleases, which catalyse the removal of a single nucleotide monophosphate (dNMP) from the end of one strand of DNA and serve as a proof-reader during DNA replication, are crucial for genome integrity. However, DHA-PPQ treatment failure has been linked to *P. falciparum* exonuclease gene alterations (Diakité et al., 2019). This mutation indicated that, recrudescence isolates from DHA-PPQ treatment failures in Cambodia had a non-synonymous single nucleotide polymorphism (SNP) E415G substitution on an exonuclease-encoding gene (Amato et al., 2017). However other researchers disagree (Boonyalai et al., 2022). Additionally, Rob W. van der Pluijm verifies treatment failure for uncomplicated *P. falciparum* malaria in Cambodia, Vietnam, and Thailand, which led to the substitution of dihydroartemisinin-piperaquine in Southern Vietnam and Northeastern Thailand for artesunate-pyronaridine. However, But in Robs' study, plasmepsin2/3 amplification status and four *Pfcrt* gene (Thr93Ser, His97Tyr, Phe145Ile, and Ile218Phe) alterations were independently linked to treatment failure (van der Pluijm et al., 2019). In Mali, two isolates out of 214 *P.*

falciparum sample exhibited the *Pfexo*-E415G mutation (Diakité et al., 2019). However, studies in Senegal (Robert et al., 2019), Sudan (Hussien et al., 2020) and the current study did not record the *Pfexonuclease*-E415G mutation in the *P. falciparum* isolates examined. The lack of *Pf-exonuclease* E415G mutation in the study area and its low prevalence on the African continent suggests that DHA-PPQ continues to be effective. However, the gene should be closely monitored.

CHAPTER FIVE

SUMMARY, NOVELTY, CONCLUSIONS AND RECOMMENDATION

Summary

Malaria prevention measures have been implemented in the Central Region, including the widespread distribution of bed nets sprayed with long-lasting insecticides. Also, the health facilities prescribe ACTs as recommended by Ghana's malaria control policy. However, discrepancies in use may influence the genetic characteristics of the parasites. This study offers details on the disease burden and genetic landscape of parasites in Ghana's Central Region. Overall, the findings of this study provide information on the presence and prevalence of *P. falciparum*, *P. vivax*, *P. ovale*, and *P. malariae* infection in the region. Thus, at present, RDT and microscopy are not conclusive for malaria diagnosis. The interplay of climatic and ecological factors sustained the persistence of malaria throughout the study sites. The malaria burden and transmission varied from district to district in the Central Region with high prevalence, especially in Assin Fosu and the Gomoa East.

The district of stay had an influence on the age group affected by malaria. However, there is a shift in the disease burden from the age group 5 years and below to the age group 5-9 years among the districts studied. The female gender is associated with high malaria prevalence in the Central Region

Previous studies have demonstrated that the COI and transmission intensity are not directly correlated. Based on this premise, malaria transmission was significantly greater in the coastal area, with CCM having a high COI. Furthermore, high parasite diversity is a characteristic of regions

with high malaria transmission. This study equally supports this assertion, as more than 50% of infected individuals carried more than one parasite genotype.

The wild-type haplotype *(CVMNK)* was often found, but *Pfcrt* gene polymorphisms associated with CQ resistance *(CVIET)* were uncommon. In this investigation, the N86Y, Y184Y, and D1246Y alleles were not found, while the Pfmdr1 N86 Y184F D1246 haplotype had the highest frequencies. The 86Y, F184, and 1246N haplotype were also identified. However, the clinical implication is not yet established. These observations suggest that there is a selective pressure affected by the high use of Artemether Lumefantrine (AL) and may affect its use as an effective choice for treatment in the near possible future.

Mutants *Pfdhfr* codon 164L as well as the *Pfdhps* K540E and 581G mutation, found to be gaining appreciable grounds in the study population should be periodically examined to keep a watch out for signs of directional selection and an increase in frequency. The haplotype conferring partial resistance to Sulfadoxine-pyrimethamine (SP) (IRNG) was the most dominant, whilst the haplotype associated with full resistance was of low frequency. In the results analysed, no super-resistant Pfdhfr-Pfdhps haplotypes were found. Nonetheless, the discovery of mutations at codons 581G and 613S/T, linked to increased resistance to sulfadoxine-pyrimethamine, foretells the emergence of highly resistant haplotypes. Thus, therapeutic studies should be conducted to assess their impact on drug efficacy and malaria control. The low frequency of parasites with SP super-resistance and quintuple mutations supports the

continued use of Sulfadoxine-pyrimethamine for Intermittent Preventive Treatment of malaria in pregnancy in Ghana.

This study showed a high prevalence of single-nucleotide polymorphism compared to the results from previous studies in the country and provides demonstrates early signs of potentially significant polymorphisms in the propeller domain of *Pfkelch13* gene. The number and distribution of polymorphisms in different geographic regions varies, which illustrates how various eco-geographic factors influence the dynamics of malaria infection transmission. The study recorded evidence of artemisinin-resistant SNPs as recorded in South East Asia (SEA). The ACTs, which include Artesunate-Amodiaquine (ASAQ) and Artemether Lumefantrine (AL), were accepted in Ghana for the treatment of uncomplicated malaria in 2006 and 2009, respectively. Therefore, drug pressure due to uncontrolled use (prescription or self-medication) might have selected resistant parasites over time. Thus, evidence of a potential spontaneous mutation in the study area includes the discovery of the M476I validated marker associated with drug resistance in SEA, the N537D and V568A candidate markers linked to delayed clearance of *P. falciparum* post ACT treatment, and new single-nucleotide polymorphisms. However, human migration cannot be ruled out. Also, as artemisinin resistance may select for various markers in different places, it is important to establish the relationship between our findings and resistance to the drug.

Novelty

Pfkelch13 SNPs S485G, I405V, I406T, I684V, K420R, M460I, M460V, E426K, F614S and G591V were identified. However, to the best of my

knowledge, no reports of these polymorphormisms exist in other endemic nations.

Conclusions

This thesis aimed at establishing the incidence of *Plasmodium* species, as well as the genetic variety of *P. falciparum* mutations linked to antimalarial medications from infected individuals.

The specific objectives were to investigate the prevalence and epidemiological risk factors of malaria in selected sites, to determine the parasite population structure and transmission intensity between the coastal and forest zones using the Clonal complexity within the ecological zones, to model the relationship between suspected drug resistance haplotypes and independent factors and to identify the antimalarial drug-associated alleles in the *Pfcrt, Pfmdr1, Pfdhps, Pfdhfr, Pfexonuclease,* and *Pfkelch13* genes. The study revealed malaria epidemiology in five districts, set in two different ecological zones in the Central Region of Ghana. These were, Abura- Asebu-Kwamankese, and the Cape Coast Metropolis in the coastal area whereas the Agona Swedru Municipality, Assin Central Municipality, and the Gomoa East District, located in the forest zones.

Irrespective of the district of stay, the infection prevalence was higher among the female gender. There is also diversity in the districts with regards to the age burden of malaria however an overall shift from age group less than to age group 5-9 years was observed. Age group 5-9 years and less than 5 years, antenatal cases, dry season, coastal ecology, and district of stay were all malaria risk associated.

The four *Plasmodium* species found in the blood samples were *P. falciparum, P. malariae, P. ovale, and P. vivax.* 98.9% of malaria infections were caused by *P. falciparum*, whereas mixed infections were caused by *P. malariae, P. ovale, and P. vivax.*

Both ecology and season influence malaria endemicity in the region, with the forest zones and rainy season contributing a high prevalence of parasite positivity compared to the coastal whilst the forest zone and dry season recorded higher complexity of infection even though not statistically significant.

The results show a symbiotic complexity in natural infection recording a high prevalence of polyclonal infections. According to the findings, the CVMNK haplotype, which has CQ sensitive allele K76, is quite common. The NFD, IRN, and SGKAA-resistant haplotypes were also quite common in the Pfmdr1, Pfdhfr, and Pfdhps genes, respectively. The data equally predicts the emergence of SP full and super resistant haplotypes with the identification of K540, A581G, and A613S/T SNPs, particularly among polyclonal infections. Mutations in the *Pfkelch13* gene linked to ACT resistance were the validated marker M476I, and candidate markers N537D and V568A. The mutations A578V, P413L, A676D, and A578S were observed at high frequency even though these mutations are not associated with ACT resistance. Resistance markers were associated with the district of stay, age group 14-19, and dry season in the *Pfkelch13*, *Pfcrt*, and *Pfdhfr* genes respectively.

Recommendations

1. The finding of *P. vivax* and the frequent misidentification of parasites other than falciparum by microscopy argues for the employment of

more sensitive malaria diagnostic tools for the determination of the true burden of non-falciparum parasites in the region. Further studies may reveal more about *P. vivax* in the region.

2. The high malaria prevalence and ecological diversity position the Central Region of Ghana as a hotspot for malaria and shows that national and regional averages do not predict local burden. The region should be considered for district-level climate-related control interventions (especially Assin central and Gomoa East districts) targeting the forest zones all year round and the coastal zones during the dry season.
3. A shift of the disease burden from the age group 5 years and below to the age group 5-9 years suggests that a scale-up of such effective interventions (such as distribution of ITNs to the > 5 y group) can lead to adequate control and management of malaria incidence and mortality.
4. Malaria surveillance should analyse data on gender to help understand the gender-specific changes in malaria transmission, infection, and illness are influenced by biological and socio-behavioral variables.
5. The frequency of polygenic infections common to all districts indicates high and stable transmission. Further studies are needed to establish the mechansisms that umder parasite coinfection, ascertain the importance of co-transmission to malaria sustenance, and how it influences parasite drug resistance.
6. It is necessary to conduct in vivo research/ Treatment Efficacy Studies (TES) to verify the efficacy of Chloroquine *Pfcrt* wild type haplotype

(CVNMK), evaluate the efficacy of AL or AS-MQ among *Pfmdr1NFD* haplotypes, and help identify the correlation of codon 184F and D1246N with partner drug resistance in Ghana. Further tests on *Pfplasmepsin2* copy numbers would confirm the state of Piperaquine.

7. The finding indicates the emergence of *P. falciparum* ACT (M476I) and SP (K540E, A581G, and A618S/T) resistant markers at low frequencies. Molecular surveillance operable at the regional level in Ghana, rational use of antimalarial against *P. falciparum* strains, genome editing, and gene manipulation experiments targeting different markers (e.g. ATG18, coronin, and *pf*ap2mu) might help control drug resistance, elucidate the role of the mutant alleles and identify the source of ACT resistance in isolates since molecular markers differ in different countries as observed with CQ. A study covering a larger selection of sites in the region may reveal unidentified diversities of interest among the haplotypes observed.

REFERENCES

Abate, A., Assefa, M., & Golassa, L. (2022). Five-Year Trend of Malaria Prevalence in Mojo Town, Central Ethiopia: Shifting Burden of the Disease and Its Implication for Malaria Elimination: A Retrospective Study. *Infection and Drug Resistance, 15*, 455.

Abuaku, B., Amoah, L. E., Peprah, N. Y., Asamoah, A., Amoako, E. O., Donu, D., . . . Malm, K. L. (2021). Malaria parasitaemia and mRDT diagnostic performances among symptomatic individuals in selected health care facilities across Ghana. *BMC public health, 21*(1), 1-11.

Abugri, J., Ansah, F., Asante, K. P., Opoku, C. N., Amenga-Etego, L. A., & Awandare, G. A. (2018). Prevalence of chloroquine and antifolate drug resistance alleles in Plasmodium falciparum clinical isolates from three areas in Ghana. *AAS Open Research, 1*.

Abukari, Z., Okonu, R., Nyarko, S. B., Lo, A. C., Dieng, C. C., Salifu, S. P., . . . Amoah, L. E. (2019). The diversity, multiplicity of infection and population structure of P. falciparum parasites circulating in asymptomatic carriers living in high and low malaria transmission settings of Ghana. *Genes, 10*(6), 434.

Achol, E., Ochaya, S., Malinga, G. M., Edema, H., & Echodu, R. (2019). High prevalence of Pfmdr-1 N86 and D1246 genotypes detected among febrile malaria outpatients attending Lira Regional Referral Hospital, Northern Uganda. *BMC research notes, 12*(1), 1-4.

Adam, R., Mukhtar, M. M., Abubakar, U. F., Damudi, H. A., Muhammad, A., & Ibrahim, S. S. (2021). Polymorphism Analysis of pfmdr1 and pfcrt from Plasmodium falciparum Isolates in Northwestern Nigeria

Revealed the Major Markers Associated with Antimalarial Resistance. *Diseases*, *9*(1), 6.

Adamu, A., Jada, M. S., Haruna, H. M. S., Yakubu, B. O., Ibrahim, M. A., Balogun, E. O., . . . Kita, K. (2020). Plasmodium falciparum Multidrug Resistance Gene-1 N86Y-Y184F-D1246Y Polymorphisms in Northern Nigeria: Implications for the continued use of artemether-lumefantrine in the region. *Plasmodium Falciparum Multidrug Resistance Gene-1 N86Y-Y184F-D1246Y Polymorphisms in Northern Nigeria: Implications for the Continued Use of Artemether-Lumefantrine in the Region*, *19*(1).

Adeshina, O. O., Nyame, S., Milner, J., Milojevic, A., & Asante, K. P. (2022). Barriers and facilitators to nationwide implementation of the malaria vaccine in Ghana. *Health policy and planning*, czac077.

Adjah, J., Fiadzoe, B., Ayanful-Torgby, R., & Amoah, L. E. (2018). Seasonal variations in Plasmodium falciparum genetic diversity and multiplicity of infection in asymptomatic children living in southern Ghana. *BMC Infectious Diseases*, *18*(1), 1-10.

Afutu, L. L., Boampong, J. N., & Quashie, N. B. (2021). High Prevalence of Molecular Markers of Plasmodium falciparum Resistance to Sulphadoxine–Pyrimethamine in Parts of Ghana: A Threat to ITPTp-SP? *Journal of Tropical Pediatrics*, *67*(1), fmaa120.

Agyeman-Budu, A., Brown, C., Adjei, G., Adams, M., Dosoo, D., Dery, D., . . . Owusu-Agyei, S. (2013). Trends in multiplicity of Plasmodium falciparum infections among asymptomatic residents in the middle belt of Ghana. *Malaria journal*, *12*, 1-6.

Ahenkorah, B., Nsiah, K., Baffoe, P., Ofosu, W., Gyasi, C., & Owiredu, E.-W. (2020). Parasitic infections among pregnant women at first antenatal care visit in northern Ghana: a study of prevalence and associated factors. *PLoS One, 15*(7), e0236514.

Ajogbasile, F. V., Oluniyi, P. E., Kayode, A. T., Akano, K. O., Adegboyega, B. B., Philip, C., . . . Wammanda, R. D. (2022). Molecular profiling of the artemisinin resistance Kelch 13 gene in Plasmodium falciparum from Nigeria. *PLoS One, 17*(2), e0264548.

Akpan, G. E., Adepoju, K. A., Oladosu, O. R., & Adelabu, S. A. (2018). Dominant malaria vector species in Nigeria: Modelling potential distribution of Anopheles gambiae sensu lato and its siblings with MaxEnt. *PLoS One, 13*(10), e0204233.

Al-Mekhlafi, H. M., Madkhali, A. M., Abdulhaq, A. A., Atroosh, W. M., Ghzwani, A. H., Zain, K. A., . . . Alharazi, T. H. (2022). Polymorphism analysis of pfmdr1 gene in Plasmodium falciparum isolates 11 years post-adoption of artemisinin-based combination therapy in Saudi Arabia. *Scientific reports, 12*(1), 1-13.

Alifrangis, M., Dalgaard, M. B., Lusingu, J. P., Vestergaard, L. S., Staalsoe, T., Jensen, A. T., . . . Warhurst, D. C. (2006). Occurrence of the Southeast Asian/South American SVMNT haplotype of the chloroquine-resistance transporter gene in Plasmodium falciparum in Tanzania. *Journal of infectious diseases, 193*(12), 1738-1741.

Allotey, N.-K., Osae, K., Mohammed, W., Kotoh, A. M., & Kenu, E. (2021). Are there variations in hotspots in clinical malaria in pregnancy and neonatal malaria? Evidence from Ghana.

Almaw, A., Yimer, M., Alemu, M., & Tegegne, B. (2022). Prevalence of malaria and associated factors among symptomatic pregnant women attending antenatal care at three health centers in north-west Ethiopia. *PLoS One, 17*(4), e0266477.

Amambua-Ngwa, A., Amenga-Etego, L., Kamau, E., Amato, R., Ghansah, A., Golassa, L., . . . Maïga-Ascofaré, O. (2019). Major subpopulations of Plasmodium falciparum in sub-Saharan Africa. *Science, 365*(6455), 813-816.

Amaratunga, C., Lim, P., Suon, S., Sreng, S., Mao, S., Sopha, C., . . . Fairhurst, R. M. (2016). Dihydroartemisinin–piperaquine resistance in Plasmodium falciparum malaria in Cambodia: a multisite prospective cohort study. *The Lancet infectious diseases, 16*(3), 357-365.

Amato, R., Lim, P., Miotto, O., Amaratunga, C., Dek, D., Pearson, R. D., . . . Suon, S. (2017). Genetic markers associated with dihydroartemisinin–piperaquine failure in Plasmodium falciparum malaria in Cambodia: a genotype–phenotype association study. *The Lancet infectious diseases, 17*(2), 164-173.

Amenga-Etego, L. N., Asoala, V., Agongo, G., Jacob, C., Goncalves, S., Awandare, G. A., . . . Kwiatkowski, D. (2021). Temporal evolution of sulfadoxine-pyrimethamine resistance genotypes and genetic diversity in response to a decade of increased interventions against Plasmodium falciparum in northern Ghana. *Malaria journal, 20*(1), 1-11.

Amoah, L., E.,, Abukari, Z., Dawson-Amoah, M. E., Dieng, C. C., Lo, E., & Afrane, Y. A. (2021). Population structure and diversity of Plasmodium falciparum in children with asymptomatic malaria living

in different ecological zones of Ghana. *BMC Infectious Diseases, 21*(1), 1-13.

Amoah, L. E., Acquah, F. K., Ayanful-Torgby, R., Oppong, A., Abankwa, J., Obboh, E. K., . . . Theisen, M. (2018). Dynamics of anti-MSP3 and Pfs230 antibody responses and multiplicity of infection in asymptomatic children from southern Ghana. *Parasites & vectors, 11*(1), 1-9.

Amoah, L. E., Donu, D., Abuaku, B., Ahorlu, C., Arhinful, D., Afari, E., . . . Koram, K. A. (2019). Probing the composition of Plasmodium species contained in malaria infections in the Eastern region of Ghana. *BMC public health, 19*(1), 1-11.

Aninagyei, E., Acheampong, D. O., Ampomah, P., Egyir-Yawson, A., & Boye, A. (2020). Characterization of putative drug resistant markers in Plasmodium falciparum isolated in Ghanaian blood donors. *International Journal of Infectious Diseases, 101*, 432.

Aninagyei, E., Stella., S.-G., Boye, A., Egyir-Yawson, A., & Acheampong, D. O. (2019). Evaluating 18s-rRNA LAMP and selective whole genome amplification (sWGA) assay in detecting asymptomatic Plasmodium falciparum infections in blood donors. *Malaria journal, 18*(1), 1-11.

Ansah, P. O., Ansah, N. A., Malm, K., Awuni, D., Peprah, N., Dassah, S., . . . Awoonor-Williams, J. (2021). Evaluation of pilot implementation of seasonal malaria chemoprevention on morbidity in young children in Northern Sahelian Ghana. *Malaria journal, 20*(1), 1-10.

Apinjoh, T. O., Mugri, R. N., Miotto, O., Chi, H. F., Tata, R. B., Anchang-Kimbi, J. K., . . . Jacob, C. (2017). Molecular markers for artemisinin and partner drug resistance in natural Plasmodium falciparum populations following increased insecticide treated net coverage along the slope of mount Cameroon: cross-sectional study. *Infectious diseases of poverty*, *6*(1), 1-10.

Apinjoh, T. O., Ouattara, A., Titanji, V. P., Djimde, A., & Amambua-Ngwa, A. (2019). Genetic diversity and drug resistance surveillance of Plasmodium falciparum for malaria elimination: is there an ideal tool for resource-limited sub-Saharan Africa? *Malaria journal*, *18*(1), 1-12.

Ariey, F., Witkowski, B., Amaratunga, C., Beghain, J., Langlois, A.-C., Khim, N., . . . Ménard , D. (2014). A molecular marker of artemisinin-resistant Plasmodium falciparum malaria. *Nature*, *505*(7481), 50-55.

Asante, K. P., Adjei, G., Enuameh, Y., & Owusu-Agyei, S. (2016). RTS, S malaria vaccine development: progress and considerations for postapproval introduction. *Vaccine: Development and Therapy*, *6*, 25-32.

Asare, K. K., Africa, J., Mbata, J., & Opoku, Y. K. (2021). The emergence of chloroquine-sensitive Plasmodium falciparum is influenced by selected communities in some parts of the Central Region of Ghana. *Malaria journal*, *20*(1), 1-9.

Asare, K. K., Boampong, J. N., Afoakwah, R., Ameyaw, E. O., Sehgal, R., & Quashie, N. B. (2014). Use of proscribed chloroquine is associated with an increased risk of pfcrt T76 mutation in some parts of Ghana. *Malaria journal*, *13*(1), 1-8.

Ashley, E. A., Dhorda, M., Fairhurst, R. M., Amaratunga, C., Lim, P., Suon, S., . . . White, N. J. (2014). Spread of artemisinin resistance in Plasmodium falciparum malaria. *New England Journal of Medicine, 371*(5), 411-423.

Ashley, E. A., Pyae Phyo, A., & Woodrow, C. J. (2018). Malaria. The Lancet, 391(10130), 1608-1621. doi:https://doi.org/10.1016/S0140-6736(18)30324-6.

Asua, V., Conrad, M. D., Aydemir, O., Duvalsaint, M., Legac, J., Duarte, E., . . . Yeka, A. (2021). Changing prevalence of potential mediators of aminoquinoline, antifolate, and artemisinin resistance across Uganda. *The Journal of infectious diseases, 223*(6), 985-994.

Auburn, S., & Barry, A. E. (2017). Dissecting malaria biology and epidemiology using population genetics and genomics. *International journal for parasitology, 47*(2-3), 77-85.

Awine, T., Malm, K., Bart-Plange, C., & Silal, S. P. (2017). Towards malaria control and elimination in Ghana: challenges and decision making tools to guide planning. *Global health action, 10*(1), 1381471.

Awine, T., Malm, K., Peprah, N. Y., & Silal, S. P. (2018). Spatio-temporal heterogeneity of malaria morbidity in Ghana: analysis of routine health facility data. *PLoS One, 13*(1), e0191707.

Awosolu, O. B., Yahaya, Z. S., Haziqah, M. T. F., Simon-Oke, I. A., & Fakunle, C. (2021). A cross-sectional study of the prevalence, density, and risk factors associated with malaria transmission in urban communities of Ibadan, Southwestern Nigeria. *Heliyon, 7*(1), e05975.

Ayanful-Torgby, R., Quashie, N. B., Boampong, J. N., Williamson, K. C., & Amoah, L. E. (2018). Seasonal variations in Plasmodium falciparum parasite prevalence assessed by varying diagnostic tests in asymptomatic children in southern Ghana. *PLoS One, 13*(6), e0199172.

Aydemir, O., Janko, M., Hathaway, N. J., Verity, R., Mwandagalirwa, M. K., Tshefu, A. K., . . . Bailey, J. A. (2018). Drug-resistance and population structure of Plasmodium falciparum across the Democratic Republic of Congo using high-throughput molecular inversion probes. *The Journal of infectious diseases, 218*(6), 946-955.

Baghbanzadeh, M., Kumar, D., Yavasoglu, S. I., Manning, S., Hanafi-Bojd, A. A., Ghasemzadeh, H., . . . Haque, U. (2020). Malaria epidemics in India: Role of climatic condition and control measures. *Science of The Total Environment, 712*, 136368.

Bahl, A., Brunk, B., Crabtree, J., Fraunholz, M. J., Gajria, B., Grant, G. R., . . . Labo, P. (2003). PlasmoDB: the Plasmodium genome resource. A database integrating experimental and computational data. *Nucleic acids research, 31*(1), 212-215.

Baird, J. K. (2022). African Plasmodium vivax malaria improbably rare or benign. *Trends in Parasitology*.

Bajoga, U. A., Balarabe, H. S., Olufemi, A. A., Dalhat, M. M., Sule, I. B., Ibrahim, M. S., . . . Ajayi, I. O. O. (2019). Trend of malaria cases in Kaduna state using routine surveillance data, 2011-2015. *The Pan African Medical Journal, 32*(Suppl 1).

Bakhiet, A. M., Abdelraheem, M. H., Kheir, A., Omer, S., Gismelseed, L., Abdel-Muhsin, A.-M. A., . . . Al Rubkhi, M. (2019). Evolution of Plasmodium falciparum drug resistance genes following artemisinin combination therapy in Sudan. *Transactions of the Royal Society of Tropical Medicine and Hygiene, 113*(11), 693-700.

Balikagala, B., Fukuda, N., Ikeda, M., Katuro, O. T., Tachibana, S.-I., Yamauchi, M., . . . Kimura, E. (2021). Evidence of artemisinin-resistant malaria in Africa. *New England Journal of Medicine, 385*(13), 1163-1171.

Balogun, S. T., Sandabe, U. K., Sodipo, O. A., Okon, K. O., & Akanmu, A. O. (2021). Single Nucleotide Polymorphisms of Pfdhfr and Pfdhps Genes: Implications for Malaria Prophylactic Strategies in Maiduguri, Northeast Nigeria. *Journal of tropical medicine, 2021*.

Basco, L. K., de Pécoulas, P. E., Wilson, C. M., Le Bras, J., & Mazabraud, A. (1995). Point mutations in the dihydrofolate reductase-thymidylate synthase gene and pyrimethamine and cycloguanil resistance in. *Molecular and biochemical parasitology, 69*, 135-138.

Basuki, S., Risamasu, P. M., Ariami, P., Riyanto, S., Hidayat, A., Susilowati, D., . . . Uemura, H. (2018). Origins and spread of novel genetic variants of sulfadoxine–pyrimethamine resistance in Plasmodium falciparum isolates in Indonesia. *Malaria journal, 17*(1), 1-14.

Behrens, H. M., Schmidt, S., Peigney, D., May, J., Maïga-Ascofaré, O., & Spielmann, T. (2022). Moderate ART resistance mutations with low fitness cost in malaria parasites from Africa. *bioRxiv*.

Bello, A. B. (2021). Risk Status of Malaria Based on Socio-Demographic and Environmental Risk Factors in Two Communities in Lagos, Nigeria.

Benié, E., Silué, K. D., Ding, X. C., Yeo, I., Assamoi, J., Tuo, K., . . . Assi, S.-B. (2022). Accuracy of a rapid diagnosis test, microscopy and loop-mediated isothermal amplification in the detection of asymptomatic Plasmodium infections in Korhogo, Northern Côte d'Ivoire. *Malaria journal*, *21*(1), 1-10.

Benjamin, G., Inabo, H., Doko, M., & Busayo, O. (2021). Assessment of Malaria Prevalence and Haemoglobin Genotypes among Patients Attending Selected Hospitals in the Three Senatorial Districts of Kaduna State, Nigeria. *Nigerian Journal of Microbiology*, *35*(1), 5568-5575.

Bennink, S., Kiesow, M. J., & Pradel, G. (2016). The development of malaria parasites in the mosquito midgut. *Cellular microbiology*, *18*(7), 905-918.

Bergmann, C., van Loon, W., Habarugira, F., Tacoli, C., Jäger, J. C., Savelsberg, D., . . . Ndoli, J. (2021). Increase in kelch 13 polymorphisms in Plasmodium falciparum, southern Rwanda. *Emerging infectious diseases*, *27*(1), 294.

Berzosa, P., de Lucio, A., Romay-Barja, M., Herrador, Z., González, V., García, L., . . . Valladares, B. (2018). Comparison of three diagnostic methods (microscopy, RDT, and PCR) for the detection of malaria parasites in representative samples from Equatorial Guinea. *Malaria journal*, *17*(1), 1-12.

Bhatt, S., Weiss, D., Cameron, E., Bisanzio, D., Mappin, B., Dalrymple, U., . . . Eckhoff, P. (2015). The effect of malaria control on Plasmodium falciparum in Africa between 2000 and 2015. *Nature, 526*(7572), 207-211.

Birhanu, Z., Yihdego, Y. Y.-e., & Yewhalaw, D. (2018). Quantifying malaria endemicity in Ethiopia through combined application of classical methods and enzyme-linked immunosorbent assay: an initial step for countries with low transmission initiating elimination programme. *Malaria journal, 17*(1), 1-14.

Birnbaum, J., Scharf, S., Schmidt, S., Jonscher, E., Hoeijmakers, W. A. M., Flemming, S., . . . Bergmann, B. (2020). A Kelch13-defined endocytosis pathway mediates artemisinin resistance in malaria parasites. *Science, 367*(6473), 51-59.

Blasco, B., Leroy, D., & Fidock, D. A. (2017). Antimalarial drug resistance: linking Plasmodium falciparum parasite biology to the clinic. *Nature medicine, 23*(8), 917-928.

Boonyalai, N., Kirativanich, K., Thamnurak, C., Praditpol, C., Vesely, B. A., Wojnarski, M., . . . Waters, N. C. (2022). A single point mutation in the Plasmodium falciparum 3′-5′ exonuclease does not alter piperaquine susceptibility. *Malaria journal, 21*(1), 1-14.

Boukoumba, F. M., Lekana-Douki, J. B., Matsiegui, P. B., Moukodoum, D. N., Adegnika, A. A., & Oyegue-Liabagui, S. L. (2021). High prevalence of genotypes associated with sulfadoxine/pyrimethamine resistance in the rural area of Fougamou, Gabon. *Journal of Global Antimicrobial Resistance, 25*, 181-186.

Breglio, K. F., Amato, R., Eastman, R., Lim, P., Sa, J. M., Guha, R., . . . McKnight, C. (2018). A single nucleotide polymorphism in the Plasmodium falciparum atg18 gene associates with artemisinin resistance and confers enhanced parasite survival under nutrient deprivation. *Malaria journal*, *17*(1), 1-16.

Brown, C. A., Pappoe-Ashong, P. J., Duah, N., Ghansah, A., Asmah, H., Afari, E., & Koram, K. A. (2021). High frequency of the Duffy-negative genotype and absence of Plasmodium vivax infections in Ghana. *Malaria journal*, *20*(1), 1-7.

Brown, T. S., Jacob, C. G., Silva, J. C., Takala-Harrison, S., Djimdé, A., Dondorp, A. M., . . . Cummings, M. P. (2015). Plasmodium falciparum field isolates from areas of repeated emergence of drug resistant malaria show no evidence of hypermutator phenotype. *Infection, Genetics and Evolution*, *30*, 318-322.

Bushman, M., Antia, R., Udhayakumar, V., & de Roode, J. C. (2018). Within-host competition can delay evolution of drug resistance in malaria. *PLoS biology*, *16*(8), e2005712.

Buyon, L. E., Elsworth, B., & Duraisingh, M. T. (2021). The molecular basis of antimalarial drug resistance in Plasmodium vivax. *International Journal for Parasitology: Drugs and Drug Resistance*, *16*, 23-37.

Bwire, G. M., Ngasala, B., Mikomangwa, W. P., Kilonzi, M., & Kamuhabwa, A. A. (2020). Detection of mutations associated with artemisinin resistance at k13-propeller gene and a near complete return of chloroquine susceptible falciparum malaria in Southeast of Tanzania. *Scientific reports*, *10*(1), 1-7.

Carlton, J. M., Volkman, S. K., Uplekar, S., Hupalo, D. N., Alves, J. M. P., Cui, L., . . . Escalante, A. A. (2015). Population genetics, evolutionary genomics, and genome-wide studies of malaria: a view across the International Centers of Excellence for Malaria Research. *The American journal of tropical medicine and hygiene, 93*(3_Suppl), 87-98.

CDC. (2019). Malaria [Online]. Centers for Disease Control and Prevention. Available:https://www.cdc.gov/malaria/about/disease.html [Accessed 11/02/2019].

Chang, H.-H., Worby, C. J., Yeka, A., Nankabirwa, J., Kamya, M. R., Staedke, S. G., . . . Jeffreys, A. E. (2017). THE REAL McCOIL: A method for the concurrent estimation of the complexity of infection and SNP allele frequency for malaria parasites. *PLoS computational biology, 13*(1), e1005348.

Chaponda, E. B., Mharakurwa, S., Michelo, C., Bruce, J., Chandramoha, D., & Matthew Chico, R. (2021). Sulfadoxine-pyrimethamine parasitological efficacy against Plasmodium falciparum among pregnant women and molecular markers of resistance in Zambia: an observational cohort study. *Malaria journal, 20*(1), 1-10.

Chatterjee, M., Ganguly, S., Saha, P., Guha, S. K., & Maji, A. K. (2017). Polymorphisms in pfdhfr and pfdhps genes after five years of artemisinin combination therapy (ACT) implementation from urban Kolkata, India. *Infection, Genetics and Evolution, 53*, 155-159.

Chaturvedi, R., Chhibber-Goel, J., Verma, I., Gopinathan, S., Parvez, S., & Sharma, A. (2021). Geographical spread and structural basis of sulfadoxine-pyrimethamine drug-resistant malaria parasites. *International journal for parasitology, 51*(7), 505-525.

Chauvin, P., Menard, S., Iriart, X., Nsango, S. E., Tchioffo, M. T., Abate, L., . . . Berry, A. (2015). Prevalence of Plasmodium falciparum parasites resistant to sulfadoxine/pyrimethamine in pregnant women in Yaoundé, Cameroon: emergence of highly resistant pfdhfr/pfdhps alleles. *Journal of Antimicrobial Chemotherapy, 70*(9), 2566-2571.

Chen, J.-H., Fen, J., & Zhou, X.-N. (2021). From 30 million to zero malaria cases in China: lessons learned for China–Africa collaboration in malaria elimination. In: BioMed Central.

Chenet, S. M., Okoth, S. A., Kelley, J., Lucchi, N., Huber, C. S., Vreden, S., . . . Adhin, M. R. (2017). Molecular profile of malaria drug resistance markers of Plasmodium falciparum in Suriname. *Antimicrobial agents and chemotherapy, 61*(7), e02655-02616.

Cheng, B., Htoo, S. N., Mhote, N. P. P., & Davison, C. M. (2021). A systematic review of factors influencing participation in two types of malaria prevention intervention in Southeast Asia. *Malaria journal, 20*(1), 1-9.

Chidimatembue, A., Svigel, S. S., Mayor, A., Aíde, P., Nhama, A., Nhamussua, L., . . . Enosse, S. (2021). Molecular surveillance for polymorphisms associated with artemisinin-based combination therapy resistance in Plasmodium falciparum isolates collected in Mozambique, 2018. *Malaria journal, 20*(1), 1-9.

Chitnumsub, P., Jaruwat, A., Talawanich, Y., Noytanom, K., Liwnaree, B., Poen, S., & Yuthavong, Y. (2020). The structure of Plasmodium falciparum hydroxymethyldihydropterin pyrophosphokinase - dihydropteroate synthase reveals the basis of sulfa resistance. *The FEBS Journal, 287*(15), 3273-3297.

Cisse, M., Awandare, G. A., Soulama, A., Tinto, H., Hayette, M.-P., & Guiguemdé, R. T. (2017). Recent uptake of intermittent preventive treatment during pregnancy with sulfadoxine–pyrimethamine is associated with increased prevalence of Pfdhfr mutations in Bobo-Dioulasso, Burkina Faso. *Malaria journal*, *16*(1), 1-8.

Cockburn, I. A., & Seder, R. A. (2018). Malaria prevention: from immunological concepts to effective vaccines and protective antibodies. *Nature immunology, 19*(11), 1199-1211.

Cohee, L. M., & Laufer, M. K. (2017). Malaria in children. *Pediatric Clinics, 64*(4), 851-866.

Collins, K. A., Ceesay, S., Drammeh, S., Jaiteh, F. K., Guery, M. A., Lanke, K., . . . D'alessandro, U. (2022). A cohort study on the duration of Plasmodium falciparum infections during the dry season in The Gambia. *The Journal of infectious diseases*, *226*(1), 128-137.

Conrad, M. D., & Rosenthal, P. J. (2019). Antimalarial drug resistance in Africa: the calm before the storm? *The Lancet infectious diseases, 19*(10), e338-e351.

Coulibaly, D., Guindo, B., Niangaly, A., Maiga, F., Konate, S., Kodio, A., . . . Traore, K. (2021). A decline and age shift in malaria incidence in rural Mali following implementation of seasonal malaria chemoprevention

and indoor residual spraying. *The American journal of tropical medicine and hygiene, 104*(4), 1342.

Dakorah, M. P., Aninagyei, E., Attoh, J., Adedia, D., Tettey, C. O., Kyei-Barffour, I., & Acheampong, D. O. (2022). Ecological and seasonal variations and other factors associated with clinical malaria in the Central Region of Ghana: A cross-sectional study. *Journal of Infection and Public Health*.

Dara, A., Dogga, S. K., Rop, J., Ouologuem, D., Tandina, F., Talman, A. M., . . . Lawniczak, M. K. (2022). Tackling malaria transmission at a single cell level in an endemic setting in sub-Saharan Africa. *Nature Communications, 13*(1), 1-3.

Das, M. K., Chetry, S., Kalita, M. C., & Dutta, P. (2016). Evidence of triple mutant Pfdhps ISGNGA haplotype in Plasmodium falciparum isolates from North-east India: an analysis of sulfadoxine resistant haplotype selection. *Genomics Data, 10*, 144-150.

Das, S., Datta, R., Beng, T. Y., & Kiromat, M. (2022). Building a gender responsive framework for malaria elimination in Asia-Pacific. *The Lancet Regional Health–Western Pacific, 22*.

Das, S., Kérah-Hinzoumbé, C., Kebféné, M., Srisutham, S., Nagorngar, T.-Y., Saralamba, N., . . . Guérin, P. J. (2022). Molecular surveillance for operationally relevant genetic polymorphisms in Plasmodium falciparum in Southern Chad, 2016–2017. *Malaria journal, 21*(1), 1-10.

de Cola, M. A., Sawadogo, B., Richardson, S., Ibinaiye, T., Traoré, A., Compaoré, C. S., . . . Rassi, C. (2022). Impact of seasonal malaria chemoprevention on prevalence of malaria infection in malaria indicator surveys in Burkina Faso and Nigeria. *BMJ Global Health, 7*(5), e008021.

de Laurent, Z. R., Chebon, L. J., Ingasia, L. A., Akala, H. M., Andagalu, B., Ochola-Oyier, L. I., & Kamau, E. (2018). Polymorphisms in the K13 gene in Plasmodium falciparum from different malaria transmission areas of Kenya. *The American journal of tropical medicine and hygiene, 98*(5), 1360.

Dembele, L., Aniweh, Y., Diallo, N., Sogore, F., Sangare, C. P. O., Haidara, A. S., . . . Campo, B. (2021). Plasmodium malariae and Plasmodium falciparum comparative susceptibility to antimalarial drugs in Mali. *Journal of Antimicrobial Chemotherapy, 76*(8), 2079-2087.

DePina, A. J., Dia, A. K., de Ascenção Soares Martins, A., Ferreira, M. C., Moreira, A. L., Leal, S. V., . . . da Moura, A. J. F. (2019). Knowledge, attitudes and practices about malaria in Cabo Verde: a country in the pre-elimination context. *BMC public health, 19*(1), 1-14.

Dhorda, M., Amaratunga, C., & Dondorp, A. M. (2021). Artemisinin and multidrug-resistant Plasmodium falciparum–a threat for malaria control and elimination. *Current opinion in infectious diseases, 34*(5), 432.

Diakité, S. A., Traoré, K., Sanogo, I., Clark, T. G., Campino, S., Sangaré, M., . . . Doucouré, F. (2019). A comprehensive analysis of drug resistance

molecular markers and Plasmodium falciparum genetic diversity in two malaria endemic sites in Mali. *Malaria journal, 18*(1), 1-9.

Djaman, J. A., Olefongo, D., Ako, A. B., Roman, J., Ngane, V. F., Basco, L. K., & Tahar, R. (2017). Molecular epidemiology of malaria in Cameroon and Cote d'Ivoire. XXXI. Kelch 13 propeller sequences in Plasmodium falciparum isolates before and after implementation of artemisinin-based combination therapy. *The American journal of tropical medicine and hygiene, 97*(1), 222.

Djimdé, A., Doumbo, O. K., Cortese, J. F., Kayentao, K., Doumbo, S., Diourté, Y., . . . Nomura, T. (2001). A molecular marker for chloroquine-resistant falciparum malaria. *New England Journal of Medicine, 344*(4), 257-263.

Dongho, G. B. D., Gunalan, K., L'Episcopia, M., Paganotti, G. M., Menegon, M., Sangong, R. E., . . . Sobze, M. S. (2021). Plasmodium vivax infections detected in a large number of febrile Duffy-negative Africans in Dschang, Cameroon. *The American journal of tropical medicine and hygiene, 104*(3), 987.

Dorkenoo, A. M., Yehadji, D., Agbo, Y. M., Layibo, Y., Agbeko, F., Adjeloh, P., . . . Ringwald, P. (2016). Therapeutic efficacy trial of artemisinin-based combination therapy for the treatment of uncomplicated malaria and investigation of mutations in k13 propeller domain in Togo, 2012–2013. *Malaria journal, 15*(1), 1-9.

Doutoum, A. A., Doungous, D. M., Gondimo, E. G., Laougangta, R. D., Adoum, A., Garandi, B., & Njintang, N. Y. (2019). Prévalence et facteurs des risques associés au paludisme chez les patients de

l'Hôpital Provincial d'Abéché (Tchad). *International Journal of Biological and Chemical Sciences, 13*(4), 1995-2004.

Duah, N. O., Matrevi, S. A., Quashie, N. B., Abuaku, B., & Koram, K. A. (2016). Genetic diversity of Plasmodium falciparum isolates from uncomplicated malaria cases in Ghana over a decade. *Parasites & vectors, 9*(1), 1-8.

Duah, N. O., Wilson, M. D., Ghansah, A., Abuaku, B., Edoh, D., Quashie, N. B., & Koram, K. A. (2007). Mutations in Plasmodium falciparum chloroquine resistance transporter and multidrug resistance genes, and treatment outcomes in Ghanaian children with uncomplicated malaria. *Journal of Tropical Pediatrics, 53*(1), 27-31.

Duffy, C. W., Assefa, S. A., Abugri, J., Amoako, N., Owusu-Agyei, S., Anyorigiya, T., . . . Awandare, G. A. (2015). Comparison of genomic signatures of selection on Plasmodium falciparum between different regions of a country with high malaria endemicity. *BMC genomics, 16*(1), 1-11.

Dun-Dery, F., Meissner, P., Beiersmann, C., Kuunibe, N., Winkler, V., Albrecht, J., & Müller, O. (2021). Uptake challenges of intermittent preventive malaria therapy among pregnant women and their health care providers in the Upper West Region of Ghana: a mixed-methods study. *Parasite Epidemiology and Control, 15*, e00222.

Duraisingh, M. T., & Cowman, A. F. (2005). Contribution of the pfmdr1 gene to antimalarial drug-resistance. *Acta tropica, 94*(3), 181-190.

Early, A. M., Daniels, R. F., Farrell, T. M., Grimsby, J., Volkman, S. K., Wirth, D. F., . . . Neafsey, D. E. (2019). Detection of low-density Plasmodium falciparum infections using amplicon deep sequencing. *Malaria journal, 18*(1), 1-13.

Ebel, E. R., Reis, F., Petrov, D. A., & Beleza, S. (2021). Historical trends and new surveillance of Plasmodium falciparum drug resistance markers in Angola. *Malaria journal, 20*(1), 1-9.

Eboumbou Moukoko, C. E., Huang, F., Nsango, S. E., Kojom Foko, L. P., Ebong, S. B., Epee Eboumbou, P., . . . Ayong, L. (2019). K-13 propeller gene polymorphisms isolated between 2014 and 2017 from Cameroonian Plasmodium falciparum malaria patients. *PLoS One, 14*(9), e0221895.

Ehrlich, H. Y., Bei, A. K., Weinberger, D. M., Warren, J. L., & Parikh, S. (2021). Mapping partner drug resistance to guide antimalarial combination therapy policies in sub-Saharan Africa. *Proceedings of the National Academy of Sciences, 118*(29), e2100685118.

Ejigu, B. A., & Wencheko, E. (2021). Spatial Prevalence and Determinants of Malaria among under-five Children in Ghana. *MedRxiv*.

Endo, N., Yamana, T., & Eltahir, E. A. (2017). Impact of climate change on malaria in Africa: a combined modelling and observational study. *The Lancet, 389*, S7.

Escobar, C., Pateira, S., Lobo, E., Lobo, L., Teodosio, R., Dias, F., . . . Nogueira, F. (2015). Polymorphisms in Plasmodium falciparum K13-propeller in Angola and Mozambique after the introduction of the ACTs. *PLoS One, 10*(3), e0119215.

Fagbemi, K. A., Adebusuyi, S. A., Nderu, D., Adedokun, S. A., Pallerla, S. R., Amoo, A. O., . . . Ojurongbe, O. (2020). Analysis of sulphadoxine–pyrimethamine resistance-associated mutations in Plasmodium falciparum isolates obtained from asymptomatic pregnant women in Ogun State, Southwest Nigeria. *Infection, Genetics and Evolution, 85*, 104503.

Fidock, D. A., Nomura, T., Talley, A. K., Cooper, R. A., Dzekunov, S. M., Ferdig, M. T., . . . Su, X.-z. (2000). Mutations in the P. falciparum digestive vacuole transmembrane protein PfCRT and evidence for their role in chloroquine resistance. *Molecular cell, 6*(4), 861-871.

Fitri, L. E., Widaningrum, T., Endharti, A. T., Prabowo, M. H., Winaris, N., & Nugraha, R. Y. B. (2022). Malaria diagnostic update: From conventional to advanced method. *Journal of Clinical Laboratory Analysis, 36*(4), e24314.

Fola, A. A., Harrison, G. A., Hazairin, M. H., Barnadas, C., Hetzel, M. W., Iga, J., . . . Barry, A. E. (2017). Higher complexity of infection and genetic diversity of Plasmodium vivax than Plasmodium falciparum across all malaria transmission zones of Papua New Guinea. *The American journal of tropical medicine and hygiene, 96*(3), 630-641.

Fontecha, G., Pinto, A., Archaga, O., Betancourth, S., Escober, L., Henríquez, J., . . . Mejía, R. E. (2021). Assessment of Plasmodium falciparum anti-malarial drug resistance markers in pfcrt and pfmdr1 genes in isolates from Honduras and Nicaragua, 2018–2021. *Malaria journal, 20*(1), 1-8.

Forson, A. O., Hinne, I. A., Dhikrullahi, S. B., Sraku, I. K., Mohammed, A. R., Attah, S. K., & Afrane, Y. A. (2022). The resting behavior of malaria vectors in different ecological zones of Ghana and its implications for vector control. *Parasites & vectors, 15*(1), 1-14.

Fukuda, N., Tachibana, S.-I., Ikeda, M., Sakurai-Yatsushiro, M., Balikagala, B., Katuro, O. T., . . . Yatsushiro, S. (2021). Ex vivo susceptibility of Plasmodium falciparum to antimalarial drugs in Northern Uganda. *Parasitology international, 81*, 102277.

Galinsky, K., Valim, C., Salmier, A., de Thoisy, B., Musset, L., Legrand, E., . . . Daniels, R. F. (2015). COIL: a methodology for evaluating malarial complexity of infection using likelihood from single nucleotide polymorphism data. *Malaria journal, 14*(1), 1-9.

Gama, B. E., Pereira-Carvalho, G. A., Lutucuta Kosi, F. J., Almeida de Oliveira, N. K., Fortes, F., Rosenthal, P. J., . . . de Fátima Ferreira-da-Cruz, M. (2010). Plasmodium falciparum isolates from Angola show the StctVMNT haplotype in the pfcrt gene. *Malaria journal, 9*(1), 1-6.

Gansané, A., Moriarty, L. F., Ménard, D., Yerbanga, I., Ouedraogo, E., Sondo, P., . . . Tapsoba, M. (2021). Anti-malarial efficacy and resistance monitoring of artemether-lumefantrine and dihydroartemisinin-piperaquine shows inadequate efficacy in children in Burkina Faso, 2017–2018. *Malaria journal, 20*(1), 1-12.

Garrido-Cardenas, J. A., González-Cerón, L., Manzano-Agugliaro, F., & Mesa-Valle, C. (2019). Plasmodium genomics: an approach for learning about and ending human malaria. *Parasitology Research, 118*(1), 1-27.

Gaye, A., Sy, M., Ndiaye, T., Siddle, K. J., Park, D. J., Deme, A. B., . . . Neafsey, D. E. (2020). Amplicon deep sequencing of kelch13 in Plasmodium falciparum isolates from Senegal. *Malaria journal, 19*(1), 1-8.

Gnagne, A. P., Konate, A., Bedia-Tanoh, A. V., Amiah-Droh, M., Menan, H. I. E., N'Guetta, A. S.-P., & Yavo, W. (2019). Dynamics of Plasmodium falciparum genetic diversity among asymptomatic and symptomatic children in three epidemiological areas in Cote d'Ivoire. *Pathogens and global health, 113*(3), 133-142.

Grais, R. F., Laminou, I. M., Woi-Messe, L., Makarimi, R., Bouriema, S. H., Langendorf, C., . . . Fandeur, T. (2018). Molecular markers of resistance to amodiaquine plus sulfadoxine–pyrimethamine in an area with seasonal malaria chemoprevention in south central Niger. *Malaria journal, 17*(1), 1-9.

Grant, J., Sagara, I., Zongo, I., Cairns, M., Yerbanga, R. S., Diarra, M., . . . Sompougdou, F. (2022). Impact of seasonal RTS, S/AS01E vaccination plus seasonal malaria chemoprevention on the nutritional status of children in Burkina Faso and Mali. *Malaria journal, 21*(1), 1-15.

Greenwood, B. (2017). New tools for malaria control–using them wisely. *Journal of Infection, 74*, S23-S26.

GSS. (2017). Ghana Statistical Service (GSS) Ghana Health Service (GHS) and International Coach Federation (ICF).Ghana Malaria Indicator Survey 2016. Accra, Ghana, Rockville, Maryland, USA: GSS, GHS and ICF.

GSS. (2020). *Ghana Statistical Service-GSS,National Malaria Control Programme-NMCP, National Public Health Reference Laboratory-NPHRL, ICF,Ghana Malaria Indicator Survey 2019*. Retrieved from Accra, Ghana, and Rockville, Maryland, USA: https://www.dhsprogram.com/pubs/pdf/MIS35/MIS35.pdf

GSS. (2021). Ghana Statistical Service, "2021 Population and Housing Census: Press release on provisional report," 2021.],.

Gueye, N. S. G., Ntoumi, F., Vouvoungui, C., Kobawila, S. C., Nkombo, M., Mouanga, A. M., . . . , & Koukouikila-Koussounda, F. (2018). Plasmodium falciparum merozoite protein-1 genetic diversity and multiplicity of infection in isolates from Congolese children consulting in a pediatric hospital in Brazzaville. *Acta Tropica, 183*, *78-83. doi:https://doi.org/10.1016/j.actatropica.04.012*.

Guo, Y., Ding, X., Shen, Y., Lyon, G. J., & Wang, K. (2015). SeqMule: automated pipeline for analysis of human exome/genome sequencing data. *Scientific reports*, *5*(1), 14283.

Gupta, H., Macete, E., Bulo, H., Salvador, C., Warsame, M., Carvalho, E., . . . Enosse, S. (2018). Drug-resistant polymorphisms and copy numbers in Plasmodium falciparum, Mozambique, 2015. *Emerging infectious diseases*, *24*(1), 40.

Haileselassie, W., Parker, D. M., Taye, B., David, R. E., Zemene, E., Lee, M.-C., . . . Tadele, G. (2022). Burden of malaria, impact of interventions and climate variability in Western Ethiopia: an area with large irrigation based farming. *BMC public health*, *22*(1), 1-11.

He, X., Zhong, D., Zou, C., Pi, L., Zhao, L., Qin, Y., . . . Wu, Y. (2021). Unraveling the complexity of imported malaria infections by amplicon deep sequencing. *Frontiers in cellular and infection microbiology*, 826.

He, Y., Campino, S., Diez Benavente, E., Warhurst, D. C., Beshir, K. B., Lubis, I., . . . Sun, X. (2019). Artemisinin resistance-associated markers in Plasmodium falciparum parasites from the China-Myanmar border: predicted structural stability of K13 propeller variants detected in a low-prevalence area. *PLoS One, 14*(3), e0213686.

Heinemann, M., Phillips, R. O., Vinnemeier, C. D., Rolling, C. C., Tannich, E., & Rolling, T. (2020). High prevalence of asymptomatic malaria infections in adults, Ashanti Region, Ghana, 2018. *Malaria journal, 19*(1), 1-7.

Hemming-Schroeder, E., Umukoro, E., Lo, E., Fung, B., Tomás-Domingo, P., Zhou, G., . . . Githeko, A. (2018). Impacts of antimalarial drugs on Plasmodium falciparum drug resistance markers, Western Kenya, 2003–2015. *The American journal of tropical medicine and hygiene, 98*(3), 692.

Hemming-Schroeder, E., Zhong, D., Kibret, S., Chie, A., Lee, M.-C., Zhou, G., . . . Yan, G. (2021). Microgeographic epidemiology of malaria parasites in an irrigated area of western Kenya by deep amplicon sequencing. *The Journal of infectious diseases, 223*(8), 1456-1465.

Hinne, I. A., Attah, S. K., Mensah, B. A., Forson, A. O., & Afrane, Y. A. (2021). Larval habitat diversity and Anopheles mosquito species distribution in different ecological zones in Ghana. *Parasites & vectors*, *14*(1), 1-14.

Howes, R. E., Battle, K. E., Mendis, K. N., Smith, D. L., Cibulskis, R. E., Baird, J. K., & Hay, S. I. (2016). Global epidemiology of Plasmodium vivax. *The American journal of tropical medicine and hygiene*, *95*(6 Suppl), 15.

Huang, B., Wang, Q., Deng, C., Wang, J., Yang, T., Huang, S., . . . Li, G. (2016). Prevalence of crt and mdr-1 mutations in Plasmodium falciparum isolates from Grande Comore island after withdrawal of chloroquine. *Malaria journal*, *15*(1), 1-9.

Hussain, M., Fatah, A., Akhtar, W., Javeed, F., Kashif, M., & Ahmed, W. (2021). Malarial infection in population of district Umerkot, Sindh & its effects on hematological parameters. *Pakistan Journal of Pathology*, *32*(1), 15-18.

Hussien, M., Abdel Hamid, M. M., Elamin, E. A., Hassan, A. O., Elaagip, A. H., Salama, A. H. A., . . . Mohamed, A. O. (2020). Antimalarial drug resistance molecular makers of Plasmodium falciparum isolates from Sudan during 2015–2017. *PLoS One*, *15*(8), e0235401.

Ibrahim, A., Diez Benavente, E., Nolder, D., Proux, S., Higgins, M., Muwanguzi, J., . . . Nosten, F. (2020). Selective whole genome amplification of Plasmodium malariae DNA from clinical samples reveals insights into population structure. *Scientific reports*, *10*(1), 1-11.

Ikeda, M., Kaneko, M., Tachibana, S.-I., Balikagala, B., Sakurai-Yatsushiro, M., Yatsushiro, S., . . . Hashimoto, M. (2018). Artemisinin-resistant Plasmodium falciparum with high survival rates, Uganda, 2014–2016. *Emerging infectious diseases*, *24*(4), 718.

Ikegbunam, M. N., Anagu, L. O., Duru, C., Nworu, C. S., Esimone, C. O., & Ojurongbe, O. (2022). Genetic diversity and allelic frequency of antigenic markers in Plasmodium falciparum isolates from Nnewi district in Nigeria. *The Journal of Infection in Developing Countries*, *16*(03), 557-563.

Imwong, M., Dhorda, M., Tun, K. M., Thu, A. M., Phyo, A. P., Proux, S., . . . Duanguppama, J. (2020). Molecular epidemiology of resistance to antimalarial drugs in the Greater Mekong subregion: an observational study. *The Lancet infectious diseases*, *20*(12), 1470-1480.

Imwong, M., Suwannasin, K., Kunasol, C., Sutawong, K., Mayxay, M., Rekol, H., & ., e. a. (2017). The spread of artemisinin-resistant Plasmodium falciparum in the Greater Mekong subregion: a molecular epidemiology observational study. *Lancet Infect Dis.;17:491–7*.

Ingasia, L. A., Cheruiyot, J., Okoth, S. A., Andagalu, B., & Kamau, E. (2016). Genetic variability and population structure of Plasmodium falciparum parasite populations from different malaria ecological regions of Kenya. *Infection, Genetics and Evolution*, *39*, 372-380.

Ippolito, M. M., Moser, K. A., Kabuya, J.-B. B., Cunningham, C., & Juliano, J. J. (2021). Antimalarial drug resistance and implications for the WHO global technical strategy. *Current Epidemiology Reports*, *8*(2), 46-62.

Kaingona-Daniel, E. P., Gomes, L. R., Gama, B. E., Almeida-de-Oliveira, N. K., Fortes, F., Ménard, D., . . . Ferreira-da-Cruz, M. d. F. (2016). Low-grade sulfadoxine–pyrimethamine resistance in Plasmodium falciparum parasites from Lubango, Angola. *Malaria journal, 15*(1), 1-6.

Kamau, E., Campino, S., Amenga-Etego, L., Drury, E., Ishengoma, D., Johnson, K., . . . Mead, D. (2015). K13-propeller polymorphisms in Plasmodium falciparum parasites from sub-Saharan Africa. *The Journal of infectious diseases, 211*(8), 1352-1355.

Kar, N. P., Chauhan, K., Nanda, N., Kumar, A., Carlton, J. M., & Das, A. (2016). Comparative assessment on the prevalence of mutations in the Plasmodium falciparum drug-resistant genes in two different ecotypes of Odisha state, India. *Infection, Genetics and Evolution, 41*, 47-55.

Kar, N. P., Kumar, A., Singh, O. P., Carlton, J. M., & Nanda, N. (2014). A review of malaria transmission dynamics in forest ecosystems. *Parasites & vectors, 7*(1), 1-12.

Kateera, F., Nsobya, S. L., Tukwasibwe, S., Hakizimana, E., Mutesa, L., Mens, P. F., . . . Kumar, N. (2016). Molecular surveillance of Plasmodium falciparum drug resistance markers reveals partial recovery of chloroquine susceptibility but sustained sulfadoxine-pyrimethamine resistance at two sites of different malaria transmission intensities in Rwanda. *Acta tropica, 164*, 329-336.

Kayiba, N. K., Yobi, D. M., Tchakounang, V. R. K., Mvumbi, D. M., Kabututu, P. Z., Devleesschauwer, B., . . . Mvumbi, G. L. (2021). Evaluation of the usefulness of intermittent preventive treatment of

malaria in pregnancy with sulfadoxine-pyrimethamine in a context with increased resistance of Plasmodium falciparum in Kingasani Hospital, Kinshasa in the Democratic Republic of Congo. *Infection, Genetics and Evolution, 94*, 105009.

Khan, W., Rahman, A. U., Shafiq, S., Ihsan, H., & Khan, K. (2019). Malaria prevalence in Malakand district, the north western region of Pakistan. *JPMA, 69*(946).

Kheang, S. T., Sovannaroth, S., Ek, S., Chy, S., Chhun, P., Mao, S., . . . Kak, N. (2017). Prevalence of K13 mutation and Day-3 positive parasitaemia in artemisinin-resistant malaria endemic area of Cambodia: a cross-sectional study. *Malaria journal, 16*(1), 1-3.

Koepfli, C., & Mueller, I. (2017). Malaria epidemiology at the clone level. *Trends in Parasitology, 33*(12), 974-985.

Koita, O. A., Sangaré, L., Sango, H. A., Dao, S., Keita, N., Maiga, M., . . . Traoré, K. (2012). Effect of seasonality and ecological factors on the prevalence of the four malaria parasite species in northern Mali. *Journal of tropical medicine, 2012*.

Kombate, G., Gmakouba, W., Scott, S., Azianu, K. A., Ekouevi, D. K., & van der Sande, M. A. (2022). Regional heterogeneity of malaria prevalence and associated risk factors among children under five in Togo: evidence from a national malaria indicators survey. *Malaria journal, 21*(1), 1-11.

Kori, L. D., Valecha, N., & Anvikar, A. R. (2018). Insights into the early liver stage biology of Plasmodium. *Journal of Vector Borne Diseases, 55*(1), 9.

Kozycki, C. T., Umulisa, N., Rulisa, S., Mwikarago, E. I., Musabyimana, J. P., Habimana, J. P., . . . Krogstad, D. J. (2017). False-negative malaria rapid diagnostic tests in Rwanda: impact of Plasmodium falciparum isolates lacking hrp2 and declining malaria transmission. *Malaria journal*, *16*(1), 1-11.

Kuesap, J., Suphakhonchuwong, N., Kalawong, L., & Khumchum, N. (2022). Molecular Markers for Sulfadoxine/Pyrimethamine and Chloroquine Resistance in Plasmodium falciparum in Thailand. *The Korean Journal of Parasitology*, *60*(2), 109.

Kyere-Boateng, R., & Marek, M. V. (2021). Analysis of the social-ecological causes of deforestation and forest degradation in Ghana: Application of the DPSIR framework. *Forests*, *12*(4), 409.

Lamptey, H., Ofori, M. F., Kusi, K. A., Adu, B., Owusu-Yeboa, E., Kyei-Baafour, E., . . . Quakyi, I. A. (2018). The prevalence of submicroscopic Plasmodium falciparum gametocyte carriage and multiplicity of infection in children, pregnant women and adults in a low malaria transmission area in Southern Ghana. *Malaria journal*, *17*(1), 1-12.

Le, P. V., Kumar, P., Ruiz, M. O., Mbogo, C., & Muturi, E. J. (2019). Predicting the direct and indirect impacts of climate change on malaria in coastal Kenya. *PLoS One*, *14*(2), e0211258.

Leichty, A. R., & Brisson, D. (2014). Selective whole genome amplification for resequencing target microbial species from complex natural samples. *Genetics*, *198*(2), 473-481.

Lestari, R. A., Lusiyana, N., & Nurochmah, F. S. (2020). *The Distribution of Malaria with Seasonal in Kokap Yogyakarta 2012-2017.* Paper presented at the E3S Web of Conferences.

Lo, E., Hemming-Schroeder, E., Yewhalaw, D., Nguyen, J., Kebede, E., Zemene, E., . . . Zhou, G. (2017). Transmission dynamics of co-endemic Plasmodium vivax and P. falciparum in Ethiopia and prevalence of antimalarial resistant genotypes. *PLoS neglected tropical diseases, 11*(7), e0005806.

Lu, F., Culleton, R., Zhang, M., Ramaprasad, A., von Seidlein, L., Zhou, H., . . . Wang, W. (2017). Emergence of indigenous artemisinin-resistant Plasmodium falciparum in Africa. *New England Journal of Medicine, 376*(10), 991-993.

Lucchi, N. W., Okoth, S. A., Komino, F., Onyona, P., Goldman, I. F., Ljolje, D., . . . Kariuki, S. (2015). Increasing prevalence of a novel triple-mutant dihydropteroate synthase genotype in Plasmodium falciparum in western Kenya. *Antimicrobial agents and chemotherapy, 59*(7), 3995-4002.

Lumb, V., Das, M. K., Singh, N., Dev, V., Wajihullah, & Sharma, Y. D. (2009). Characteristics of genetic hitchhiking around dihydrofolate reductase gene associated with pyrimethamine resistance in Plasmodium falciparum isolates from India. *Antimicrobial agents and chemotherapy, 53*(12), 5173-5180.

Maghendji-Nzondo, S., Kouna, L.-C., Mourembou, G., Boundenga, L., Imboumy-Limoukou, R.-K., Matsiegui, P.-B., . . . Toure-Ndouo, F. (2016). Malaria in urban, semi-urban and rural areas of southern of

Gabon: comparison of the Pfmdr 1 and Pfcrt genotypes from symptomatic children. *Malaria journal*, *15*(1), 1-9.

Mahamar, A., Sumner, K. M., Levitt, B., Freedman, B., Traore, A., Barry, A., . . . Attaher, O. (2022). Effect of three years' seasonal malaria chemoprevention on molecular markers of resistance of Plasmodium falciparum to sulfadoxine-pyrimethamine and amodiaquine in Ouelessebougou, Mali. *Malaria journal*, *21*(1), 1-8.

Maiga, H., Grivoyannis, A., Sagara, I., Traore, K., Traore, O. B., Tolo, Y., . . . Sanogo, K. (2021). Selection of pfcrt K76 and pfmdr1 N86 coding alleles after uncomplicated malaria treatment by artemether-lumefantrine in Mali. *International journal of molecular sciences*, *22*(11), 6057.

Mairet-Khedim, M., Leang, R., Marmai, C., Khim, N., Kim, S., Ke, S., . . . Chy, S. (2021). Clinical and in vitro resistance of Plasmodium falciparum to artesunate-amodiaquine in Cambodia. *Clinical Infectious Diseases*, *73*(3), 406-413.

MalariaGEN. (2021). Genomic Epidemiology Network (Online) Aavailable at https://www.malariagen.net/projects/pf3k (Accessed 25-05-21).

Mama, A., Ahiabor, C., Tornyigah, B., Frempong, N. A., Kusi, K. A., Adu, B., . . . Ofori, M. F. (2022). Intermittent preventive treatment in pregnancy with sulfadoxine–pyrimethamine and parasite resistance: cross-sectional surveys from antenatal care visit and delivery in rural Ghana. *Malaria journal*, *21*(1), 1-9.

Maniga, J. N., Akinola, S. A., Odoki, M., Odda, J., & Adebayo, I. A. (2021). Limited Polymorphism in Plasmodium falciparum Artemisinin Resistance Kelch13-Propeller Gene Among Clinical Isolates from Bushenyi District, Uganda. *Infection and Drug Resistance, 14*, 5153.

Mathieu, L. C., Cox, H., Early, A. M., Mok, S., Lazrek, Y., Paquet, J.-C., . . . Udhayakumar, V. (2020). Local emergence in Amazonia of Plasmodium falciparum k13 C580Y mutants associated with in vitro artemisinin resistance. *Elife, 9*, e51015.

Matrevi, S. A., Opoku-Agyeman, P., Quashie, N. B., Bruku, S., Abuaku, B., & Koram, K. A. (2019). Plasmodium falciparum kelch propeller polymorphisms in clinical isolates from Ghana from 2007 to 2016. . *Antimicrob Agents Chemother.;63:e00802-e819.*

Matrevi, S. A., Tandoh, K. Z., Bruku, S., Opoku-Agyeman, P., Adams, T., Ennuson, N. A., . . . Koram, K. A. (2022). Novel pfk13 polymorphisms in Plasmodium falciparum population in Ghana. *Scientific reports, 12*(1), 1-9.

Mayengue, P. I., Niama, R. F., Kouhounina Batsimba, D., Malonga-Massanga, A., Louzolo, I., Loukabou Bongolo, N. C., . . . Dossou-Yovo, L. R. (2018). No polymorphisms in K13-propeller gene associated with artemisinin resistance in Plasmodium falciparum isolated from Brazzaville, Republic of Congo. *BMC Infectious Diseases, 18*(1), 1-6.

Mayor, A., da Silva, C., Rovira-Vallbona, E., Roca-Feltrer, A., Bonnington, C., Wharton-Smith, A., . . . Guinovart, C. (2022). Prospective surveillance study to detect antimalarial drug resistance, gene deletions

of diagnostic relevance and genetic diversity of Plasmodium falciparum in Mozambique: protocol. *BMJ open, 12*(7), e063456.

Mba, T. N., Obiang, C. S., Kenguele, H. M., Nzamba, U., Mba, P. K. B., Pambo-Pambo, A. B., & Engonga, L.-C. O. (2022). Prevalence of Malaria among Patients at the Sino-Gabonese Friendship Hospital in Franceville: A Two-Year Retrospective Study.

Menard, D., & Dondorp, A. (2017). Antimalarial drug resistance: a threat to malaria elimination. *Cold Spring Harbor perspectives in medicine, 7*(7), a025619.

Ménard, D., Khim, N., Beghain, J., Adegnika, A. A., Shafiul-Alam, M., & Amodu, O. e. a. (2016). A worldwide map of Plasmodium falciparum K13-propeller polymorphisms. *N Engl J Med.;374:2453–64.*

Mensah, B., A.,, Myers-Hansen, J., Obeng Amoako., E., Opoku, M., Abuaku, B., K.,, & Ghansah, A. (2021). Prevalence and risk factors associated with asymptomatic malaria among school children: repeated cross-sectional surveys of school children in two ecological zones in Ghana. *BMC public health, 21*(1), 1-9.

Mensah, B. A., Aydemir, O., Myers-Hansen, J., Opoku, M., Hathaway, N. J., Marsh, P. W., . . . Ghansah, A. (2020). Antimalarial drug resistance profiling of Plasmodium falciparum infections in Ghana using molecular inversion probes and next-generation sequencing. *Antimicrobial agents and chemotherapy, 64*(4), e01423-01419.

Millar, S. B., & Cox-Singh, J. (2015). Human infections with Plasmodium knowlesi--zoonotic malaria. Clin Microbiol Infect, 21, 640-8.

Miller, R. H., Hathaway, N. J., Kharabora, O., Mwandagalirwa, K., Tshefu, A., Meshnick, S. R., . . . Bailey, J. A. (2017). A deep sequencing approach to estimate Plasmodium falciparum complexity of infection (COI) and explore apical membrane antigen 1 diversity. *Malaria journal, 16*(1), 1-15.

Miotto, O., Sekihara, M., Tachibana, S.-I., Yamauchi, M., Pearson, R. D., Amato, R., . . . Marfurt, J. (2020). Emergence of artemisinin-resistant Plasmodium falciparum with kelch13 C580Y mutations on the island of New Guinea. *PLoS pathogens, 16*(12), e1009133.

Mishra, N., Bharti, R. S., Mallick, P., Singh, O. P., Srivastava, B., Rana, R., . . . Valecha, N. (2016). Emerging polymorphisms in falciparum Kelch 13 gene in Northeastern region of India. *Malaria journal, 15*(1), 1-6.

Mishra, S., Bharti, P. K., Shukla, M. M., Ali, N. A., Kashyotia, S. S., Kumar, A., . . . Singh, N. (2017). Clinical and molecular monitoring of Plasmodium falciparum resistance to antimalarial drug (artesunate+ sulphadoxine-pyrimethamine) in two highly malarious district of Madhya Pradesh, central India from 2012–2014. *Pathogens and global health, 111*(4), 186-194.

Mita, T., & Jombart, T. (2015). Patterns and dynamics of genetic diversity in Plasmodium falciparum: what past human migrations tell us about malaria. *Parasitology international, 64*(3), 238-243.

Mitchell, C. L., Ngasala, B., Janko, M. M., Chacky, F., Edwards, J. K., Pence, B. W., . . . Kyaw, T. (2022). Evaluating malaria prevalence and land cover across varying transmission intensity in Tanzania using a cross-sectional survey of school-aged children. *Malaria journal, 21*(1), 1-10.

Mlugu, E. M., Minzi, O., Asghar, M., Färnert, A., Kamuhabwa, A. A., & Aklillu, E. (2020). Effectiveness of sulfadoxine–pyrimethamine for intermittent preventive treatment of malaria and adverse birth outcomes in pregnant women. *Pathogens*, *9*(3), 207.

MOH. (2009). Ministry of Health,Ghana. Anti-malaria drug policy for Ghana, 2nd revised version 2009. Ministry of Health; 2009. Available from: http://apps.who.int/medicinedocs/en/m/abstract/Js18072en/ Assessed on 16-02-2022.

MOH. (2014). Ministry of Health: Strategic Plan for Malaria Control in Ghana 2014 –2020. .

MOH. (2016). RTS,S malaria vaccine technical brief Ghana. Ghana, 74.

Mohammed, K., Gazali, S. M., Batung, E., Amoak, D., Avoka, V. A., Kansanga, M., & Luginaah, I. (2022). Spatial analysis of climatic factors and plasmodium falciparum malaria prevalence among children in Ghana. *Spatial and Spatio-temporal Epidemiology*, 100537.

Molina-Cruz, A., Zilversmit, M. M., Neafsey, D. E., Hartl, D. L., & Barillas-Mury, C. (2016). Mosquito vectors and the globalization of Plasmodium falciparum malaria. *Annual review of genetics*, *50*, 447-465.

Morgan, A. P., Brazeau, N. F., Ngasala, B., Mhamilawa, L. E., Denton, M., Msellem, M., . . . Bailey, J. A. (2020). Falciparum malaria from coastal Tanzania and Zanzibar remains highly connected despite effective control efforts on the archipelago. *Malaria journal*, *19*(1), 1-14.

Moser, K. A., Madebe, R. A., Aydemir, O., Chiduo, M. G., Mandara, C. I., Rumisha, S. F., . . . Verity, R. (2021). Describing the current status of Plasmodium falciparum population structure and drug resistance within mainland Tanzania using molecular inversion probes. *Molecular ecology*, *30*(1), 100-113.

Mukherjee, A., Bopp, S., Magistrado, P., Wong, W., Daniels, R., Demas, A., . . . Dhorda, M. (2017). Artemisinin resistance without pfkelch13 mutations in Plasmodium falciparum isolates from Cambodia. *Malaria journal*, *16*(1), 1-12.

Mulenga, M. C., Sitali, L., Ciubotariu, I. I., Hawela, M. B., Hamainza, B., Chipeta, J., & Mharakurwa, S. (2021). Decreased prevalence of the Plasmodium falciparum Pfcrt K76T and Pfmdr1 and N86Y mutations post-chloroquine treatment withdrawal in Katete District, Eastern Zambia. *Malaria journal*, *20*(1), 1-8.

Muntaka, S., & Opoku-Okrah, C. (2013). The prevalence of malaria parasitaemia and predisposition of ABO blood groups to Plasmodium falciparum malaria among blood donors at a Ghanaian Hospital. *AU Journal of Technology*, *16*(4).

Mustafa, S. O., Hamid, M. M. A., Aboud, M. A., Amin, M., Muneer, M. S., Yasin, K., . . . El Bagir, N. M. (2017). Genetic diversity and multiplicity of Plasmodium falciparum merozoite surface protein 2 in field isolates from Sudan. *F1000Research*, *6*(1790), 1790.

Nájera, J. A., González-Silva, M., & Alonso, P. L. (2011). Some lessons for the future from the Global Malaria Eradication Programme (1955–1969). *PLoS medicine*, *8*(1), e1000412.

Ndiaye, J. L. A., Ndiaye, Y., Ba, M. S., Faye, B., Ndiaye, M., Seck, A., . . . Beshir, K. (2019). Seasonal malaria chemoprevention combined with community case management of malaria in children under 10 years of age, over 5 months, in south-east Senegal: a cluster-randomised trial. *PLoS medicine, 16*(3), e1002762.

Ndong Ngomo, J. M., Mawili-Mboumba, D. P., M'Bondoukwé, N. P., Ditombi, B. M., Koumba Lengongo, J. V., Batchy Ognagosso, F. B., & Bouyou-Akotet, M. K. (2023). Drug Resistance Molecular Markers of Plasmodium falciparum and Severity of Malaria in Febrile Children in the Sentinel Site for Malaria Surveillance of Melen in Gabon: Additional Data from the Plasmodium Diversity Network African Network. *Tropical Medicine and Infectious Disease, 8*(4), 184.

Ndwiga, L., Kimenyi, K. M., Wamae, K., Osoti, V., Akinyi, M., Omedo, I., . . . Ghansah, A. (2021). A review of the frequencies of Plasmodium falciparum Kelch 13 artemisinin resistance mutations in Africa. *International Journal for Parasitology: Drugs and Drug Resistance, 16*, 155-161.

Neafsey, D. E., Taylor, A. R., & MacInnis, B. L. (2021). Advances and opportunities in malaria population genomics. *Nature Reviews Genetics, 22*(8), 502-517.

Negatu, G. A., Abebe, G. A., & Yalew, W. G. (2022). Prevalence of malaria and associated factors among malaria-suspected patients attending Hamusit health center, Northwest Ethiopia: A cross-sectional study. *Journal of parasitology research, 2022*.

Niang, M., Diop, F., Niang, O., Sadio, B. D., Sow, A., Faye, O., . . . Toure-Balde, A. (2017). Unexpected high circulation of Plasmodium vivax in asymptomatic children from Kédougou, southeastern Senegal. *Malaria journal, 16*(1), 1-10.

Niba, P. T. N., Nji, A. M., Evehe, M.-S., Ali, I. M., Netongo, P. M., Ngwafor, R., . . . Acho, F. A. (2021). Drug resistance markers within an evolving efficacy of anti-malarial drugs in Cameroon: a systematic review and meta-analysis (1998–2020). *Malaria journal, 20*(1), 1-20.

Njiro, B. J., Mutagonda, R. F., Chamani, A. T., Mwakyandile, T., Sabas, D., & Bwire, G. M. (2022). Molecular surveillance of chloroquine-resistant Plasmodium falciparum in sub-Saharan African countries after withdrawal of chloroquine for treatment of uncomplicated malaria: a systematic review. *Journal of Infection and Public Health*.

Nkhoma, S., Trevino, S., Gorena, K., Nair, S., Khoswe, S., Jett, C., . . . Terlouw, D. (2018). Resolving within-host malaria parasite diversity using single-cell sequencing. bioRxiv 391268. In.

Nkhoma, S. C., Trevino, S. G., Gorena, K. M., Nair, S., Khoswe, S., Jett, C., . . . Terlouw, D. J. (2020). Co-transmission of related malaria parasite lineages shapes within-host parasite diversity. *Cell host & microbe, 27*(1), 93-103. e104.

Nkoli Mandoko, P., Rouvier, F., Matendo Kakina, L., Moke Mbongi, D., Latour, C., Losimba Likwela, J., . . . Muepu Tshilolo, L. (2018). Prevalence of Plasmodium falciparum parasites resistant to sulfadoxine/pyrimethamine in the Democratic Republic of the Congo:

emergence of highly resistant pfdhfr/pfdhps alleles. *Journal of Antimicrobial Chemotherapy, 73*(10), 2704-2715.

Nkwescheu, A. S., Fokam, J., Tchendjou, P., Nji, A., Ngouakam, H., Andre, B. F., . . . Mbacham, W. (2015). Current practice of epidemiology in Africa: highlights of the 3rd conference of the African epidemiological association and 1st conference of the Cameroon society of epidemiology, Yaoundé, Cameroon, 2014. *Pan African Medical Journal, 21*(1).

NMCP. (2013). National Malaria Control Program. An epidemiological profile of malaria and its control in Ghana.National Malaria Control Program; 2013. Available from: https://www.linkmalaria.org/sites/www.linkmalaria.org/files/content/country/profiles/Ghana-epi-report-2014.pdf. Assessed on 23-06-2022.

NMCP. (2016). National Malaria Control Programme, Annual report 2016, Ghana Health Service.

NMCP. (2017). Ministry of Health G. NMCP: Annual Report of the National Malaria Control Programme of Ghana, 2017. .

NMCP. (2022). National Malaria Control Programme (NMCP). Country Coordinating Mechanism (CCM) of the Global Fund to Fight AIDS, Tuberculosis and Malaria. Available at: https://www.ccmghana.net/index.php/2018-2020/malaria/national-malaria-control-programme/ Assessed on 12-07-2022.

Nsanzabana, C., Ariey, F., Beck, H.-P., Ding, X. C., Kamau, E., Krishna, S., . . . Nag, S. (2018). Molecular assays for antimalarial drug resistance surveillance: a target product profile. *PLoS One, 13*(9), e0204347.

Nzoumbou-Boko, R., Panté-Wockama, C.-B. G., Ngoagoni, C., Petiot, N., Legrand, E., Vickos, U., . . . Lombart, J.-P. (2020). Molecular assessment of kelch13 non-synonymous mutations in Plasmodium falciparum isolates from Central African Republic (2017–2019). *Malaria journal, 19*(1), 1-7.

Obaldia III, N., Baro, N. K., Calzada, J. E., Santamaria, A. M., Daniels, R., Wong, W., . . . Herrera, S. (2015). Clonal outbreak of Plasmodium falciparum infection in eastern Panama. *The Journal of infectious diseases, 211*(7), 1087-1096.

Obboh, E. K., Okonu, R. E., & Amoah, L. E. (2020). Large variations in malaria parasite carriage by afebrile school children living in nearby communities in the Central Region of Ghana. *Journal of tropical medicine, 2020*.

Oboh, M. A., Faal, F., Adeniji, O. E., Correa, S., Amawu, A. U., Ogban, E., . . . Amambua-Ngwa, A. (2022). Multiple Plasmodium falciparum drug resistance polymorphisms identified in a pregnant woman with severe malaria and a concomitant spontaneous abortion in Cross River, Nigeria, West Africa. *Malaria journal, 21*(1), 1-5.

Ocan, M., Akena, D., Nsobya, S., Kamya, M. R., Senono, R., Kinengyere, A. A., & Obuku, E. (2019). K13-propeller gene polymorphisms in Plasmodium falciparum parasite population in malaria affected countries: a systematic review of prevalence and risk factors. *Malaria journal, 18*(1), 1-17.

Ofori, M. F., Kploanyi, E. E., Mensah, B. A., Dickson, E. K., Kyei-Baafour, E., Gyabaa, S., . . . Ghansah, A. (2021). Ex vivo sensitivity profile of Plasmodium falciparum clinical isolates to a panel of antimalarial drugs in Ghana 13 years after national policy change. *Infection and Drug Resistance*, *14*, 267.

Oguike, M. C., Falade, C. O., Shu, E., Enato, I. G., Watila, I., Baba, E. S., . . . Meek, S. (2016). Molecular determinants of sulfadoxine-pyrimethamine resistance in Plasmodium falciparum in Nigeria and the regional emergence of dhps 431V. *International Journal for Parasitology: Drugs and Drug Resistance*, *6*(3), 220-229.

Okell, L. C., Reiter, L. M., Ebbe, L. S., Baraka, V., Bisanzio, D., Watson, O. J., . . . Roper, C. (2018). Emerging implications of policies on malaria treatment: genetic changes in the Pfmdr-1 gene affecting susceptibility to artemether–lumefantrine and artesunate–amodiaquine in Africa. *BMJ Global Health*, *3*(5), e000999.

Okiring, J., Epstein, A., Namuganga, J. F., Kamya, E. V., Nabende, I., Nassali, M., . . . Kiwuwa, S. M. (2022). Gender difference in the incidence of malaria diagnosed at public health facilities in Uganda. *Malaria journal*, *21*(1), 1-12.

Omedo, I., Mogeni, P., Rockett, K., Kamau, A., Hubbart, C., Jeffreys, A., et al.,. (2017). Geographic-genetic analysis of Plasmodium falciparum parasite populations from surveys of primary school children in Western Kenya. *WELLCOME OPEN RES.2017 ;2:29.*

Ontoua, S. S., Kouna, L. C., Oyegue-Liabagui, S. L., Voumbo-Matoumona, D. F., Moukodoum, D. N., Imboumy-Limoukou, R. K., & Lekana-Douki, J. B. (2021). Differential Prevalences of Pfmdr1 Polymorphisms in Symptomatic and Asymptomatic Plasmodium falciparum Infections in Lastoursville: A Rural Area in East-Central Gabon. *Infection and Drug Resistance, 14*, 2873.

Orish, V. N., Ansong, J. Y., Onyeabor, O. S., Sanyaolu, A. O., Oyibo, W. A., & Iriemenam, N. C. (2016). Overdiagnosis and overtreatment of malaria in children in a secondary healthcare centre in Sekondi-Takoradi, Ghana. *Tropical Doctor, 46*(4), 191-198.

Osarfo, J., Ampofo, G. D., & Tagbor, H. (2022). Trends of malaria infection in pregnancy in Ghana over the past two decades: a review. *Malaria journal, 21*(1), 1-12.

Osborne, A., Manko, E., Takeda, M., Kaneko, A., Kagaya, W., Chan, C., . . . Campino, S. (2021). Characterizing the genomic variation and population dynamics of Plasmodium falciparum malaria parasites in and around Lake Victoria, Kenya. *Scientific reports, 11*(1), 1-11.

Osoti, V., Akinyi, M., Wamae, K., Kimenyi, K. M., de Laurent, Z., Ndwiga, L., . . . Mwandawiro, C. (2022). Targeted Amplicon Deep Sequencing for Monitoring Antimalarial Resistance Markers in Western Kenya. *Antimicrobial agents and chemotherapy, 66*(4), e01945-01921.

Otambo, W. O., Omondi, C. J., Ochwedo, K. O., Onyango, P. O., Atieli, H., Lee, M.-C., . . . Githure, J. (2022). Risk associations of submicroscopic malaria infection in lakeshore, plateau and highland areas of Kisumu County in western Kenya. *PLoS One, 17*(5), e0268463.

Otienoburu, S. D., Suay, I., Garcia, S., Thomas, N. V., Srisutham, S., Björkman, A., & Humphreys, G. S. (2019). An online mapping database of molecular markers of drug resistance in Plasmodium falciparum: the ACT Partner Drug Molecular Surveyor. *Malaria journal, 18*(1), 1-10.

Ouji, M., Augereau, J.-M., Paloque, L., & Benoit-Vical, F. (2018). Plasmodium falciparum resistance to artemisinin-based combination therapies: A sword of Damocles in the path toward malaria elimination. *Parasite, 25*.

Owusu-Ofori, A., Gadzo, D., & Bates, I. (2016). Transfusion-transmitted malaria: donor prevalence of parasitaemia and a survey of healthcare workers knowledge and practices in a district hospital in Ghana. *Malaria journal, 15*(1), 1-7.

Owusu, E., D.,A., , Brown, C., A., , Grobusch, M., P., , & Mens, P. (2017). Prevalence of Plasmodium falciparum and non-P. falciparum infections in a highland district in Ghana, and the influence of HIV and sickle cell disease. . *Malar J. ;16(1):167.*

Oyola, S. O., Ariani, C. V., Hamilton, W. L., Kekre, M., Amenga-Etego, L. N., Ghansah, A., . . . Jyothi, D. (2016). Whole genome sequencing of Plasmodium falciparum from dried blood spots using selective whole genome amplification. *Malaria journal, 15*(1), 1-12.

Pacheco, M. A., Kadakia, E. R., Chaudhary, Z., Perkins, D. J., Kelley, J., Ravishankar, S., . . . Escalante, A. A. (2019). Evolution and genetic diversity of the k13 gene associated with artemisinin delayed parasite

clearance in Plasmodium falciparum. *Antimicrobial agents and chemotherapy, 63*(8), e02550-02518.

Pearce, R. J., Pota, H., Evehe, M.-S. B., Bâ, E.-H., Mombo-Ngoma, G., Malisa, A. L., . . . Diallo, D. A. (2009). Multiple origins and regional dispersal of resistant dhps in African Plasmodium falciparum malaria. *PLoS medicine, 6*(4), e1000055.

PMI. (2017). Presidents Malaria Initiative (PMI): Malaria Operational Plan FY 2018. Ghana Statistical Service, Accra, Ghana.

PMI. (2020). U.S. President's Malaria Initiative. Ghana Malaria Operational Plan FY 2020. Available online: www.pmi.gov. Assessed on 20-05-2022.

Poirier, P., Doderer-Lang, C., Atchade, P. S., Lemoine, J.-P., de l'Isle, M.-L. C., Abou-Bacar, A., . . . Haar, E. (2016). The hide and seek of Plasmodium vivax in West Africa: report from a large-scale study in Beninese asymptomatic subjects. *Malaria journal, 15*(1), 1-9.

Pradhan, M. M., & Meherda, P. (2019). Malaria elimination drive in Odisha: hope for halting the transmission. *Journal of Vector Borne Diseases, 56*(1), 53.

Quan, H., Igbasi, U., Oyibo, W., Omilabu, S., Chen, S.-B., Shen, H.-M., . . . Zhou, X.-N. (2020). High multiple mutations of Plasmodium falciparum-resistant genotypes to sulphadoxine-pyrimethamine in Lagos, Nigeria. *Infectious diseases of poverty, 9*(1), 1-11.

Quaresima, V., Agbenyega, T., Oppong, B., Awunyo, J. A. D., Adu Adomah, P., Enty, E., . . . Castelli, F. (2021). Are malaria risk factors based on gender? a mixed-methods survey in an urban setting in Ghana. *Tropical Medicine and Infectious Disease, 6*(3), 161.

Ranjha, R., & Sharma, A. (2021). Forest malaria: the prevailing obstacle for malaria control and elimination in India. *BMJ Global Health, 6*(5), e005391.

Ravenhall, M., Benavente, E. D., Mipando, M., Jensen, A. T., Sutherland, C. J., Roper, C., . . . Phiri, K. S. (2016). Characterizing the impact of sustained sulfadoxine/pyrimethamine use upon the Plasmodium falciparum population in Malawi. *Malaria journal, 15*(1), 1-11.

Recker, M., Bull, P. C., & Buckee, C. O. (2018). Recent advances in the molecular epidemiology of clinical malaria. *F1000Research, 7*.

RHD annual report. (2018). Regional Health Directorate (RHD) Annual Report (unpublished).

Rice, B. L., Golden, C. D., Randriamady, H. J., Rakotomalala, A. A. N. A., Vonona, M. A., Anjaranirina, E. J. G., . . . Hartl, D. L. (2021). Fine-scale variation in malaria prevalence across ecological regions in Madagascar: a cross-sectional study. *BMC public health, 21*(1), 1-13.

Robert, M. G., Foguim Tsombeng, F., Gendrot, M., Diawara, S., Madamet, M., Kounta, M. B., . . . Benoit, N. (2019). Baseline Ex Vivo and Molecular Responses of Plasmodium fa lciparum Isolates to Piperaquine before Implementation of Dihydroartemisinin-Piperaquine in Senegal. *Antimicrobial agents and chemotherapy, 63*(5), e02445-02418.

Roca-Feltrer, A., Carneiro, I., Smith, L., Schellenberg, J. R. A., Greenwood, B., & Schellenberg, D. (2010). The age patterns of severe malaria syndromes in sub-Saharan Africa across a range of transmission intensities and seasonality settings. *Malaria journal*, *9*(1), 1-9.

Rodrigues, A. B. B., de Abreu-Fernandes, R., Neto, Z., Jandondo, D., Almeida-de-Oliveira, N. K., de Lavigne Mello, A. R., . . . Ferreira-da-Cruz, M. d. F. (2022). Pfkelch13 Plasmodium falciparum Mutations in Huambo, Angola. *Pathogens*, *11*(5), 554.

Rodriguez-Barraquer, I., Arinaitwe, E., Jagannathan, P., Kamya, M. R., Rosenthal, P. J., Rek, J., . . . Kilama, M. (2018). Quantification of anti-parasite and anti-disease immunity to malaria as a function of age and exposure. *Elife*, *7*, e35832.

Roh, M. E., Tessema, S. K., Murphy, M., Nhlabathi, N., Mkhonta, N., Vilakati, S., . . . Chen, A. (2019). High genetic diversity of Plasmodium falciparum in the low-transmission setting of the Kingdom of Eswatini. *The Journal of infectious diseases*, *220*(8), 1346-1354.

Roper, C., Pearce, R., Nair, S., Sharp, B., Nosten, F., & Anderson, T. (2004). Intercontinental spread of pyrimethamine-resistant malaria. *Science*, *305*(5687), 1124-1124.

Ross, L. S., & Fidock, D. A. (2019). Elucidating mechanisms of drug-resistant Plasmodium falciparum. *Cell host & microbe*, *26*(1), 35-47.

Rotejanaprasert, C., Lee, D., Ekapirat, N., Sudathip, P., & Maude, R. J. (2021). Spatiotemporal distributed lag modelling of multiple Plasmodium species in a malaria elimination setting. *Statistical Methods in Medical Research, 30*(1), 22-34.

Roux, A. T., Maharaj, L., Oyegoke, O., Akoniyon, O. P., Adeleke, M. A., Maharaj, R., & Okpeku, M. (2021). Chloroquine and sulfadoxine–pyrimethamine resistance in Sub-Saharan Africa—A review. *Frontiers in Genetics*, 773.

Russo, G., Faggioni, G., Paganotti, G. M., Djeunang Dongho, G. B., Pomponi, A., De Santis, R., . . . Vullo, V. (2017). Molecular evidence of Plasmodium vivax infection in Duffy negative symptomatic individuals from Dschang, West Cameroon. *Malaria journal, 16*(1), 1-9.

Sakzabre, D., Asiamah, E. A., Akorsu, E. E., Abaka-Yawson, A., Dika, N. D., Kwasie, D. A., . . . Osei, G. Y. (2020). Haematological profile of adults with malaria parasitaemia visiting the Volta Regional Hospital, Ghana. *Advances in Hematology, 2020*.

Sarah-Matio, E. M., Guillochon, E., Nsango, S. E., Abate, L., Ngou, C. M., Bouopda, G. A., . . . Talman, A. (2022). Genetic diversity of Plasmodium falciparum and distribution of antimalarial drug resistance mutations in symptomatic and asymptomatic infections. *Antimicrobial agents and chemotherapy, 66*(8), e00188-00122.

Sarmah, N., Sarma, K., Bhattacharyya, D., Sultan, A., Bansal, D., Singh, N., . . . Parida, P. (2017). Antifolate drug resistance: novel mutations and

haplotype distribution in dhps and dhfr from Northeast India. *Journal of biosciences, 42*(4), 531-535.

Sasu, D. D. (2022). Number of deaths from malaria in Ghana 2008-2019, by age group. State of Health, Health, Pharma & Medtech. Statista. Available at: https://www.statista.com/statistics/1173607/number-of-deaths-from-malaria-in-ghana-by-age-group/ Assessed on January 19, 2022.

Sato, S. (2021). Plasmodium—a brief introduction to the parasites causing human malaria and their basic biology. *Journal of physiological anthropology*, *40*(1), 1-13.

Sayang, C., Gausseres, M., Vernazza-Licht, N., Malvy, D., Bley, D., & Millet, P. (2009). Treatment of malaria from monotherapy to artemisinin-based combination therapy by health professionals in urban health facilities in Yaoundé, central province, Cameroon. *Malaria journal*, *8*(1), 1-6.

Schleicher, T. R., Yang, J., Freudzon, M., Rembisz, A., Craft, S., Hamilton, M., . . . Li, Y. (2018). A mosquito salivary gland protein partially inhibits Plasmodium sporozoite cell traversal and transmission. *Nature Communications*, *9*(1), 2908.

Segun, O. E., Shohaimi, S., Nallapan, M., Lamidi-Sarumoh, A. A., & Salari, N. (2020). Statistical modelling of the effects of weather factors on malaria occurrence in Abuja, Nigeria. *International journal of environmental research and public health*, *17*(10), 3474.

Short, E. E., Caminade, C., & Thomas, B. N. (2017). Climate change contribution to the emergence or re-emergence of parasitic diseases. *Infectious Diseases: Research and Treatment, 10*, 1178633617732296.

Siakwa, M., Okanlawon, F., Druye, A., Browne, E., Boampong, J., & Benyin, E. (2014). Prevalence rate of malaria parasite in donated blood for transfusion in the central region hospital, Cape Coast, Ghana. *West African Journal of Nursing, 25*(1).

Siciliano, G., Costa, G., Suárez-Cortés, P., Valleriani, A., Alano, P., & Levashina, E. A. (2020). Critical steps of Plasmodium falciparum ookinete maturation. *Frontiers in microbiology, 11*, 269.

Siddiqui, F. A., Boonhok, R., Cabrera, M., Mbenda, H. G. N., Wang, M., Min, H., . . . Miao, J. (2020). Role of Plasmodium falciparum Kelch 13 protein mutations in P. falciparum populations from northeastern Myanmar in mediating artemisinin resistance. *MBio, 11*(1), e01134-01119.

Silué, S., Dago, D. N., Kouassi, A. A., Dajuma, A., Doumbia, M., & Kéita, S. (2021). Assessing Seasonal Climate Variability Impact on the Malaria Patient's Cases in the North of Côte d'Ivoire. *European Journal of Applied Sciences–Vol, 9*(6).

Singana, B. P., Mayengue, P. I., Niama, R. F., & Ndounga, M. (2019). Genetic diversity of Plasmodium falciparum infection among children with uncomplicated malaria living in Pointe-Noire, Republic of Congo. *Pan African Medical Journal, 32*(1).

Sondo, P., Derra, K., Lefevre, T., Diallo-Nakanabo, S., Tarnagda, Z., Zampa, O., . . . Ouedraogo, J.-B. (2019). Genetically diverse Plasmodium falciparum infections, within-host competition and symptomatic malaria in humans. *Scientific reports, 9*(1), 1-9.

Soulama, I., Nébié, I., Ouédraogo, A., Gansane, A., Diarra, A., Tiono, A. B., . . . Taylor, W. R. (2009). Plasmodium falciparum genotypes diversity in symptomatic malaria of children living in an urban and a rural setting in Burkina Faso. *Malaria journal, 8*(1), 1-8.

Stokes, B. H., Dhingra, S. K., Rubiano, K., Mok, S., Straimer, J., Gnädig, N. F., . . . Ward, K. E. (2021). Plasmodium falciparum K13 mutations in Africa and Asia impact artemisinin resistance and parasite fitness. *Elife, 10*, e66277.

Surve, K. M., Kulkarni, A. S., Rathod, S. G., & Bindu, R. S. (2017). Study of haematological parameters in malaria. *Int J Res Med Sci, 5*(6), 2552-2557.

Svigel, S. S., Adeothy, A., Kpemasse, A., Houngbo, E., Sianou, A., Saliou, R., . . . Tchevoede, A. (2021). Low prevalence of highly sulfadoxine - resistant dihydropteroate synthase alleles in Plasmodium falciparum isolates in Benin. *Malaria journal, 20*(1), 1-8.

Takala-Harrison, S., Jacob, C. G., Arze, C., Cummings, M. P., Silva, J. C., Dondorp, A. M., . . . Noedl, H. (2015). Independent emergence of artemisinin resistance mutations among Plasmodium falciparum in Southeast Asia. *The Journal of infectious diseases, 211*(5), 670-679.

Tandoh, K. Z., Amenga-Etego, L., Quashie, N. B., Awandare, G., Wilson, M., & Duah-Quashie, N. O. (2021). Plasmodium falciparum malaria parasites in Ghana show signatures of balancing selection at artemisinin resistance predisposing background genes. *Evolutionary Bioinformatics, 17*, 1176934321999640.

Taylor, S. M., Parobek, C. M., DeConti, D. K., Kayentao, K., Coulibaly, S. O., Greenwood, B. M., . . . Njie, F. (2015). Absence of putative artemisinin resistance mutations among Plasmodium falciparum in sub-Saharan Africa: a molecular epidemiologic study. *The Journal of infectious diseases, 211*(5), 680-688.

Thomsen, T. T., Madsen, L. B., Hansson, H. H., Tomás, E. V., Charlwood, D., Bygbjerg, I. C., & Alifrangis, M. (2013). Rapid selection of Plasmodium falciparum chloroquine resistance transporter gene and multidrug resistance gene-1 haplotypes associated with past chloroquine and present artemether-lumefantrine use in Inhambane District, southern Mozambique. *The American journal of tropical medicine and hygiene, 88*(3), 536.

Thuy-Nhien, N., Tuyen, N. K., Tong, N. T., Vy, N. T., Thanh, N. V., Van, H. T., . . . Dolecek, C. (2017). K13 propeller mutations in Plasmodium falciparum populations in regions of malaria endemicity in Vietnam from 2009 to 2016. *Antimicrobial agents and chemotherapy, 61*(4), e01578-01516.

Tiedje, K. E., Oduro, A. R., Agongo, G., Anyorigiya, T., Azongo, D., Awine, T., . . . Day, K. P. (2017). Seasonal variation in the epidemiology of asymptomatic Plasmodium falciparum infections across two catchment

areas in Bongo District, Ghana. *The American journal of tropical medicine and hygiene, 97*(1), 199.

Tormey, W., & O'brien, P. (1993). Clinical associations of an increased transthyretin band in routine serum and urine protein electrophoresis. *Annals of clinical biochemistry, 30*(6), 550-554.

Tornyigah, B., Coppée, R., Houze, P., Kusi, K. A., Adu, B., Quakyi, I., . . . Anang, A. K. (2020). Effect of drug pressure on promoting the emergence of antimalarial-resistant parasites among pregnant women in Ghana. *Antimicrobial agents and chemotherapy, 64*(6), e02029-02019.

Torres, K., Bachman, C. M., Delahunt, C. B., Alarcon Baldeon, J., Alava, F., Gamboa Vilela, D., . . . Thompson, C. M. (2018). Automated microscopy for routine malaria diagnosis: a field comparison on Giemsa-stained blood films in Peru. *Malaria journal, 17*(1), 1-11.

Touray, A. O., Mobegi, V.A., Wamunyokoli, F., Herren, J., K.,. (2020). Diversity and Multiplicity of P. falciparum infections among asymptomatic school children in Mbita, Western Kenya. *Sci Rep. 2020 Apr 3;10(1):5924. doi: 10.1038/s41598-020-62819-w. PMID: 32246 127; PMCID: PMC7125209.*

Tuedom, A. G. B., Sarah-Matio, E. M., Moukoko, C. E. E., Feufack-Donfack, B. L., Maffo, C. N., Bayibeki, A. N., . . . Abate, L. (2021). Antimalarial drug resistance in the Central and Adamawa regions of Cameroon: Prevalence of mutations in P. falciparum crt, Pfmdr1, Pfdhfr and Pfdhps genes. *PLoS One, 16*(8), e0256343.

Tumwebaze, P. K., Katairo, T., Okitwi, M., Byaruhanga, O., Orena, S., Asua, V., . . . Ceja, F. G. (2021). Drug susceptibility of Plasmodium falciparum in eastern Uganda: a longitudinal phenotypic and genotypic study. *The Lancet Microbe*, *2*(9), e441-e449.

Tun, K. M., Imwong, M., Lwin, K. M., Win, A. A., Hlaing, T. M., Hlaing, T., . . . Woodrow, C. J. (2015). Spread of artemisinin-resistant Plasmodium falciparum in Myanmar: a cross-sectional survey of the K13 molecular marker. *The Lancet infectious diseases*, *15*(4), 415-421.

Twohig, K. A., Pfeffer, D. A., Baird, J. K., Price, R. N., Zimmerman, P. A., Hay, S. I., . . . Howes, R. E. (2019). Growing evidence of Plasmodium vivax across malaria-endemic Africa. *PLoS neglected tropical diseases*, *13*(1), e0007140.

Ugah, U. I., Alo, M. N., Owolabi, J. O., Okata-Nwali, O. D., Ekejindu, I. M., Ibeh, N., & Elom, M. O. (2017). Evaluation of the utility value of three diagnostic methods in the detection of malaria parasites in endemic area. *Malaria journal*, *16*(1), 1-8.

Umaru, M. L., & Uyaiabasi, G. N. (2015). Prevalence of malaria in patients attending the general hospital Makarfi, Makarfi Kaduna–State, North-Western Nigeria. *American journal of infectious Diseases and Microbiology*, *3*(1), 1-5.

Uwimana, A., Legrand E, Stokes BH, Ndikumana JM, Warsame M, Umulisa N, et al. Emergence and clonal expansion of in vitro artemisinin-resistant Plasmodium falciparum kelch13 R561H mutant parasites in Rwanda. (2020). Nat Med.;26:1602–8 Presents evidence, from drug

efficacy studies of ACTs in Rwanda, of the de novo emergence of pfk13-mediated artemisinin resistance.

van der Pluijm, R. W., Imwong, M., Chau, N. H., Hoa, N. T., Thuy-Nhien, N. T., Thanh, N. V., . . . Saelow, C. (2019). Determinants of dihydroartemisinin-piperaquine treatment failure in Plasmodium falciparum malaria in Cambodia, Thailand, and Vietnam: a prospective clinical, pharmacological, and genetic study. *The Lancet infectious diseases*, *19*(9), 952-961.

Völker, F., Cooper, P., Bader, O., Uy, A., Zimmermann, O., Lugert, R., & Groß, U. (2017). Prevalence of pregnancy-relevant infections in a rural setting of Ghana. *BMC pregnancy and childbirth*, *17*(1), 1-7.

Voumbo-Matoumona, D. F., Akiana, J., Madamet, M., Kouna, L. C., Lekana-Douki, J. B., & Pradines, B. (2018). High prevalence of Plasmodium falciparum antimalarial drug resistance markers in isolates from asymptomatic patients from the Republic of the Congo between 2010 and 2015. *Journal of Global Antimicrobial Resistance*, *14*, 277-283.

Wahlgren, M., Goel, S., & Akhouri, R. R. (2017). Variant surface antigens of Plasmodium falciparum and their roles in severe malaria. *Nature Reviews Microbiology*, *15*(8), 479-491.

Wamae, K., Okanda, D., Ndwiga, L., Osoti, V., Kimenyi, K. M., Abdi, A., . . . Ochola-Oyier, L. (2019). No evidence of P. falciparum K13 artemisinin conferring mutations over a 24-year analysis in Coastal Kenya, but a near complete reversion to chloroquine wild type parasites.

Warsame, M., Hassan, A. H., Hassan, A. M., Arale, A. M., Jibril, A. M., Mohamud, S. A., . . . Nada, R. A. (2017). Efficacy of artesunate+ sulphadoxine/pyrimethamine and artemether+ lumefantrine and dhfr and dhps mutations in Somalia: evidence for updating the malaria treatment policy. *Tropical Medicine & International Health, 22*(4), 415-422.

WHO. (2009). Methods for Surveillance of Antimalarial Drug Efficacy WHO. Library Cataloguing-in-Publication Data.

WHO. (2010). *Global report on antimalarial drug efficacy and drug resistance: 2000-2010*: World Health Organization.

WHO. (2015). *Guidelines for the treatment of malaria*: World Health Organization.

WHO. (2016). Global technical strategy for malaria 2016-2030. Geneva: World Health Organization

WHO. (2017). *Artemisinin and artemisinin-based combination therapy resistance: status report. Retrieved from https://apps.who.int* Retrieved from

WHO. (2017b). *World Health Organization. A framework for malaria elimination.*: World Health Organization.

WHO. (2018). World Malaria Report. *Retrieved from http://apps.who.int/iris/bitstream/handle/10665/275867/9789241565653-eng.pdf.*

WHO. (2018a). Malaria key facts. Retrieved from https://www.who.int/news-room/factsheets/detail/malaria.

WHO. (2019). World Malaria Report. Geneva, Switzerland. *World Health Organization*.

WHO. (2020). World malaria report 2020: 20 years of global progress and challenges. Geneva: Licence: CC BY-NC-SA 3.0 IGO. (Online) Available at: https://www.who.int/docs/default-source/malaria/world-malaria-reports/9789240015791-double-page-view.pdf?sfvrsn=2c24349d_5. (Accessed 21-07-2021).

WHO. (2021). World Health Organization. Malaria. Key facts of Malaria, Fact Sheets, Newsroom. Available at: https://www.who.int/news-room/fact-sheets/detail/malaria/ Assessed on 18-08-2022

Wicht, K. J., Mok, S., & Fidock, D. A. (2020). Molecular mechanisms of drug resistance in Plasmodium falciparum malaria. *Annual review of microbiology, 74*, 431.

Wong, W., Wenger, E. A., Hartl, D. L., & Wirth, D. F. (2018). Modeling the genetic relatedness of Plasmodium falciparum parasites following meiotic recombination and cotransmission. *PLoS computational biology, 14*(1), e1005923.

Xia, J., Wu, D., Wu, K., Zhu, H., Sun, L., Lin, W., . . . Zhang, H. (2020). Epidemiology of Plasmodium falciparum Malaria and Risk Factors for Severe Disease in Hubei Province, China. *The American journal of tropical medicine and hygiene, 103*(4), 1534.

Xu, C., Wei, Q., Yin, K., Sun, H., Li, J., Xiao, T., . . . Zhu, S. (2018). Surveillance of antimalarial resistance Pfcrt, Pfmdr1, and Pfkelch13 polymorphisms in African Plasmodium falciparum imported to Shandong Province, China. *Scientific reports, 8*(1), 1-9.

Yan, H., Feng, J., Yin, J.-h., Huang, F., Kong, X.-l., Lin, K.-m., . . . Cao, J.-p. (2021). High frequency mutations in pfdhfr and pfdhps of Plasmodium falciparum in response to sulfadoxine-pyrimethamine: A cross-sectional survey in returning Chinese migrants from Africa. *Frontiers in cellular and infection microbiology, 11*.

Yang, T., Yeoh, L. M., Tutor, M. V., Dixon, M. W., McMillan, P. J., Xie, S. C., . . . Ralph, S. A. (2019). Decreased K13 abundance reduces hemoglobin catabolism and proteotoxic stress, underpinning artemisinin resistance. *Cell Reports, 29*(9), 2917-2928. e2915.

Yaqoob, A., Khattak, A. A., Nadeem, M. F., Fatima, H., Mbambo, G., Ouattara, A., . . . Takala-Harrison, S. (2018). Prevalence of molecular markers of sulfadoxine–pyrimethamine and artemisinin resistance in Plasmodium falciparum from Pakistan. *Malaria journal, 17*(1), 1-9.

Yaro, J. B., Ouedraogo, A., Diarra, A., Sombié, S., Ouedraogo, Z. A., Nébié, I., . . . Lindsay, S. W. (2021). Risk factors for Plasmodium falciparum infection in pregnant women in Burkina Faso: a community-based cross-sectional survey. *Malaria journal, 20*(1), 1-13.

Ye, R., Zhang, Y., & Zhang, D. (2022). Evaluations of candidate markers of dihydroartemisinin-piperaquine resistance in Plasmodium falciparum isolates from the China–Myanmar, Thailand–Myanmar, and Thailand–Cambodia borders. *Parasites & vectors, 15*(1), 1-9.

Yobi, D. M., Kayiba, N. K., Mvumbi, D. M., Boreux, R., Kabututu, P. Z., Akilimali, P. Z., . . . Mvumbi, G. L. (2022). Biennial surveillance of Plasmodium falciparum anti-malarial drug resistance markers in

Democratic Republic of Congo, 2017 and 2019. *BMC Infectious Diseases, 22*(1), 1-9.

Yobi, D. M., Kayiba, N. K., Mvumbi, D. M., Boreux, R., Kabututu, P. Z., Situakibanza, H. N., . . . Mvumbi, G. L. (2021). Assessment of Plasmodium falciparum anti-malarial drug resistance markers in pfk13-propeller, pfcrt and pfmdr1 genes in isolates from treatment failure patients in Democratic Republic of Congo, 2018–2019. *Malaria journal, 20*(1), 1-8.

Yogavel, M., Nettleship, J. E., Sharma, A., Harlos, K., Jamwal, A., Chaturvedi, R., . . . Sharma, A. (2018). Structure of 6-hydroxymethyl-7, 8-dihydropterin pyrophosphokinase–dihydropteroate synthase from Plasmodium vivax sheds light on drug resistance. *Journal of Biological Chemistry*, *293*(39), 14962-14972.

Zhang, J., Li, N., Siddiqui, F. A., Xu, S., Geng, J., Zhang, J., & Zhang, Y. (2019). In vitro susceptibility of Plasmodium falciparum isolates from the China-Myanmar border area to artemisinins and correlation with K13 mutations. International Journal for Parasitology: Drugs and Drug Resistance, 10, 20-27. .

Zhang, Y., Xie, L., Xie, L., & Bourne, P. E. (2016). The Plasmodium falciparum drugome and its polypharmacological implications. *bioRxiv*, 042481.

Zhao, H., Pi, L., Zhao, L., Qin, Y., Zeng, W., Xiang, Z., . . . Zou, C. (2021). First Detection in West Africa of a Mutation That May Contribute to Artemisinin Resistance Plasmodium falciparum. *Frontiers in Genetics, 12*.

Zhao, L., Pi, L., Qin, Y., Lu, Y., Zeng, W., Xiang, Z., . . . Zhang, Y. (2020). Widespread resistance mutations to sulfadoxine-pyrimethamine in malaria parasites imported to China from Central and Western Africa. *International Journal for Parasitology: Drugs and Drug Resistance, 12*, 1-6.

Zhong, D., Koepfli, C., Cui, L., & Yan, G. (2018). Molecular approaches to determine the multiplicity of Plasmodium infections. *Malaria journal, 17*(1), 1-9.

Zhou, R., Yang, C., Li, S., Zhao, Y., Liu, Y., Qian, D., . . . Huang, F. (2019). Molecular surveillance of drug resistance of Plasmodium falciparum isolates imported from Angola in Henan Province, China. *Antimicrobial agents and chemotherapy, 63*(10), e00552-00519.

Zupko, R. J., Nguyen, T. D., Somé, A. F., Tran, T. N.-A., Gerardin, J., Dudas, P., . . . Ouédraogo, J.-B. (2022). Long-term effects of increased adoption of artemisinin combination therapies in Burkina Faso. *PLOS Global Public Health, 2*(2), e0000111.

APPENDICES

APPENDIX 1: Table 71: Distribution of demographic and clinical variables of the malaria by five districts in the Central Region

Covariates	Overall n (%)	Abura-Asebu-Kwamankese n (%)	Agona Swedru n (%)	Cape Coast n (%)	Assin Central n (%)	Gomoa East n (%)	*P-value*
Number of Malaria Microscopy positive test samples	409	58 (14.2)	29 (7.1)	75 (18.3)	155 (37.9)	92 (24.5)	
Species							0.157
Pf	405 (99.0)	58 (100.0)	29 (100.0)	75 (100.0)	151 (97.4)	92 (100.0)	
Pf/Po	4 (1.0)	0	0	0	4 (2.6)	0	
Age¥	19.1±16.97	3.38±1.81	3.83±1.63	3.43±1.59	2.90±1.80	3.92±1.57	
Age-group (years)							<0.001**
Less than 5	100 (24.5)	16 (27.6)	5 (17.2)	8 (10.7)	57 (36.8)	14 (15.2)	
05-9	64 (15.7)	7 (12.1)	2 (6.9)	23 (30.7)	26 (16.8)	6 (6.5)	
10-14	27 (6.6)	3 (5.2)	3 (10.3)	6 (8.0)	4 (2.6)	11 (12.0)	
15-19	41 (10.0)	6 (10.3)	3 (10.3)	9 (12.0)	17 (11.0)	6 (6.5)	
20-59	161 (39.4)	23 (39.7)	15 (51.7)	25 (33.3)	46 (29.7)	52 (56.5)	
60 plus	16 (3.9)	3 (5.2)	1 (3.5)	4 (5.3)	5 (3.2)	3 (3.3)	
Gender							0.049*
Female	248 (60.6)	45 (77.6)	18 (62.1)	41 (54.7)	87 (56.1)	57 (62.0)	
Male	161 (39.4)	13 (22.4)	11 (37.9)	34 (45.3)	68 (43.9)	35 (38.0)	

*p<0.05 and p<0.001** were considered statistically significant
¥Mean (Standard deviation)
Source: Field work, 2021

APPENDIX 1: Table 71: Distribution of demographic and clinical variables of the malaria by five districts in the Central Region … continued

Covariates	Overall n (%)	Abura-Asebu-Kwamankese n (%)	Agona Swedru n (%)	Cape Coast n (%)	Assin Central n (%)	Gomoa East n (%)	*P-value*
Level of Education (n=358)							0.001*
No education	22 (6.2)	4 (20.0)	1 (5.6)	6 (8.0)	7 (4.6)	4 (4.4)	
Preschool	77 (20.4)	6 (30.0)	4 (22.2)	9 (12.0)	48 (31.2)	10 (11.0)	
Primary	73 (20.4)	2 (10.0)	4 (22.2)	23 (30.7)	28 (18.2)	16 (17.6)	
Middle/JSS/JHS	90 (24.1)	4 (20.0)	5 (27.8)	10 (13.3)	41 (26.6)	30 (33.0)	
Secondary/SSS/SHS	61 (17.0)	3 (15.0)	3 (16.7)	15 (20.0)	20 (13.0)	20 (22.0)	
Vocational/Commercial/Technical	14 (3.9)	1 (5.0)	0	7 (9.3)	4 (2.6)	2 (2.2)	
Post-Secondary (Nursing/Teacher Training)	7 (2.0)	0	0	0	4 (2.6)	3 (3.3)	
Tertiary	14 (3.9)	0	1 (5.6)	5 (6.7)	2 (1.3)	6 (6.6)	
Use of LLITNs (n=356)							<0.001**
No	125 (35.1)	4 (20.0)	5 (29.4)	51 (68.9)	24 (15.6)	41 (45.1)	
Yes	231 (64.9)	16 (80.0)	12 (70.6)	23 (31.1)	130 (84.4)	50 (54.9)	
participants with pre-medication (n=356)							<0.001**
No	333 (93.5)	15 (75.0)	15 (88.2)	70 (94.6)	153 (99.4)	80 (87.9)	
Yes	23 (6.5)	5 (25.0)	2 (11.8)	4 (5.4)	1 (0.7)	11 (12.1)	

*p<0.05 and p<0.001** were considered statistically significant

Source: Field work, 2021

APPENDIX 1: Distribution of demographic and clinical variables of the malaria by five districts in the Central Region … continued

Covariates	Overall n (%)	Abura-Asebu-Kwamankese n (%)	Agona Swedru n (%)	Cape Coast n (%)	Assin Central n (%)	Gomoa East n (%)	P-value
Pre-medication taken							0.070
Artemisinin Combination Therapy (ACT)	6 (26.1)	2 (40.0)	1 (50.0)	1 (25.0)	1 (100.0)	1 (9.1)	
ACT Green Leaf	3 (13.0)	1 (20.0)	1 (50.0)	1 (25.0)	0	0	
Paracetamol	10 (43.5)	1 (20.0)	0	0	0	9 (81.8)	
Quinim/ACT	1 (4.4)	1 (20.0)	0	0	0	0	
Tabia Herbal	2 (8.7)	0	0	2 (50.0)	0	0	
Teedah	1 (4.4)	0	0	0	0	1 (9.1)	
Childen under five years receiving vaccination (n=96)							0.830
Not vaccinated	14 (14.6)	3 (18.6)	2 (40.0)	4 (28.6)	3 (9.1)	2 (7.1)	
Vaccinated	82 (85.4)	13 (81.3)	3 (60.0)	10 (71.4)	30 (90.9)	26 (92.9)	
Women in Reproductive Age (n=154)							
Not Pregnant	109 (70.8)	17 (73.9)	9 (64.3)	17 (63.0)	39 (73.6)	27 (73.0)	0.133
Pregnant	45 (29.2)	6 (26.1)	5 (35.7)	10 (37.0)	14 (26.4)	10 (27.0)	
Parasite count[¥]	23195.98±44924.87	30876.81±40262.46	5360.52±6966.72	28688.12±57169.44	30682.25±52875.18	6885.73±10057.78	
Seasons							<0.001
Dry	134 (32.8)	32 (55.2)	23 (79.3)	26 (34.7)	51 (32.9)	2 (2.2)	
Rainy	275 (67.2)	26 (44.8)	6 (20.7)	49 (65.3)	104 (67.1)	90 (97.8)	
Ecological zone							<0.001
Coastal	133 (32.5)	58 (100.0)	0	75 (100.0)	0	0	
Forest	276 (67.5)	0	29 (100.0)	0	155 (100.0)	92 (100.0)	

*p<0.05 and p<0.001** were considered statistically significant [¥]Mean (Standard deviation)

Source: Field work, 2021

APPENDIX 2: Amino acids and abbreviations

Amino Acid	Symbol	Abbreviation	Amino Acid	Symbol	Abbreviation
Glycerine	G	Gly	Proline	P	Pro
Alanine	A	Ala	Valine	V	Val
Leucine	L	Leu	Isoleucine	I	Ile
Methionine	M	Met	Cysteine	C	Cys
Phenylalanine	F	Phe	Tyrosine	Y	Tyr
Thypyophan	W	Trp	Histidine	H	His
Lysine	K	Lys	Arginine	R	Arg
Glutamine	Q	Gln	Asparagine	N	Asn
Glutamic Acid	E	Glu	Aspartic Acid	D	Asp
Serine	S	Ser	Threonine	T	Thr

APPENDIX 3: Sample Collection Questionnaires

This questionnaire is intended to assist the researcher to determine if there are drug resistance genes in the community and make assessment of the impact of antimalarial medications on the health status of the patient in selected communities in the central region of Ghana. The exercise is basically academic and your answers would be treated with the utmost confidentiality they deserve. Your maximum co-operation is highly anticipated.

Please tick (√) the response applicable to the client.

Do you wish to participate in this survey? ○ Yes, permission is given ○ No, I have a change of mind and choose to withdraw
For children below 5yrs: has the child been vaccinated: YES…….. NO……
A. Questionnaire Information
1. Region_____ 2. District_____ 3. Sub-District_____
4. Name of Facility_____
5. Interviewer name and number: _____
6. Date of interview: (DD/ MM / YYYY) ___ ___/ ___ /20…….
B. Demographic/Clinical Characteristics
7. Name of patient/ PATH NUMBER: _____ ___
8. Telephone number: _____
9. Gender: ○Male ○Female
10. If female is she pregnant? ○Yes ○No
11. Locality Name (where patient stays): _____
12. Age/Date of Birth: _____
13. Height / Length: _____ 14. Weight measurement in Kilograms (kg): ____
15. Blood pressure_____
16. Level of education: ○ Preschool 0 ○ Primary 1 ○ Middle/JSS/JHS 2

○ Secondary/SSS/SHS 3 ○ Vocational/Commercial/Technical 4 ○ Post-Secondary (Nursing/Teacher Training) 5 ○ Tertiary 6

17. Ethnic group: ○ Akan ○ FANTE ○ Ga/Dangme ○ Ewe ○ Assin ○ Dagbani ○ Non-Ghanaian ○ Other ethnic group (specify) …….	18. Does your household have any mosquito nets that can be used while sleeping? Yes ○ No ○ 19. How long have you had the nets? _____ 20. Does patient sleep in net? ○ Yes ○ No

21. When you got the net, was it already treated with an insecticide to kill or repel mosquitoes? ○ Yes ○ No
22. Since you got the net, has it ever been soaked or dipped in a liquid to kill or repel mosquitoes? ○ Yes ○ No
23. How many months ago was the net last soaked or dipped?
24. In the past 2 weeks has (name) taken any medicine given by a doctor or health center to treat the malaria? ○ Yes ○ No
25. What medicine was (name) given? Probe: Any other medicine?

Antimalarials:	**Antibiotic drugs:**	**Other medications:**
○ Quinine ○ Artemisinin-based Combination ○ ACT with the green leaf ○ Other antimalarial (specify)	○ Pill / Syrup ○ Injection	○ Paracetamol/ Panadol /Acetaminophen ○ Aspirin ○ Ibuprofen ○ Other (specify)

26. What is the current prescribed medication GIVEN FOR FEVER OR MALARIA DURING THIS ILLNESS?

Antimalarials:	**Antibiotic drugs:**	**Other medications:**
○ Quinine ○ Artemisinin-based	○ Pill /	○ Paracetamol/ Panadol/ ○ Acetaminophen

Combination ○ ACT with the green leaf ○ Other antimalarial (specify) ……	Syrup ○ Injection	○ Aspirin ○ Ibuprofen ○ Other (specify) ○ Herbal preparation ……
27. Malaria testing outcome. (a) RDT: …… (b) Microscopy: ……..		

APPENDIX 4: Consent form for adults

CONSENT FORM: MALARIA POSITIVE CASES ADULTS

Title of Study: Genetic characterization of anti-malaria drug resistant strains in Central region of Ghana

Institution: University of Cape Coast, Department of Biomedical Sciences

PARTICIPANT STATEMENT AND SIGNATURE

I certify that I voluntarily agree to participate in this study and that the study has been explained to me in a language I understand (FANTE, TWI, ENGLISH, OTHER…….). All my questions have also been answered satisfactorily in the language I understand. I also understand that I am free to withdraw from this study and that my withdrawal will have no negative implications on any other dealings that I have with the facilities involved as a patient patronizing the facility. A copy of the information sheet will be given to you after it has been signed or thumb-printed to keep.

Signature Participant	or	
Date:		
		Left thumbprint

Signature witness	or	
Date:		
		Left thumbprint

INVESTIGATOR STATEMENT AND SIGNATURE

I certify that the participant has been given ample time to read and learn about the study and I have fully explained to those who cannot read in the language they understand. All questions and clarifications raised by the participant have been addressed.

Signature…….

Name of ……..... for Miss Mavis Dakorah (PI)

Date ……...

APPENDIX 5: Consent form for minors

CONSENT FORM: MALARIA POSITIVE CASES- MINORS

Title of Study: Genetic characterization of anti-malaria drug resistant strains in Central region of Ghana

Institution: University of Cape Coast, Department of Biomedical Sciences

PARTICIPANT STATEMENT AND SIGNATURE

I certify that I voluntarily agree to answer the study questions on behalf of my child/ ward, that this study and that the study has been explained to me in a language I understand (FANTE, TWI, ENGLISH, OTHER…….). All my questions have also been answered satisfactorily in the language I understand. I also understand that the I am free to withdraw my child from this study and that the withdrawal of my child/ward will have no negative implications on any other dealings that I have with the facilities involved as a patient patronizing the facility. A copy of the information sheet and consent form will be given to you after it has been signed or thumb-printed to keep.

Signature Participant	or	
Date:		
		Left thumbprint

Signature witness	or	
Date:		
		Left thumbprint

INVESTIGATOR STATEMENT AND SIGNATURE

I certify that the participant has been given ample time to read and learn about the study and I have fully explained to those who cannot read in the language they understand. All questions and clarifications raised by the participant have been addressed.

Signature…….

Name of ……..... for Miss Mavis Dakorah (PI)

Date ……...

APPENDIX 6: List of District in the Central Region of Ghana

No.	Forest	No.	Coastal
1	Asikuman / Odoben / Brakwa	1	Mfantsiman
2	Upper Denkyira East	2	Effutu
3	Twifo Ati Morkwa	3	Ccm - Cape Coast North
4	Agona West	4	Ccm - Cape Coast South
5	Ajumako-Enyan-Esiam	5	Gomoa West
6	Assin Fosu	6	Komenda Edina Eguafo Abirem
7	Upper Denkyira West	7	Abura / Asebu / Kwamankese
8	Twifo Lower Denkyira	8	Ekumfi
9	Assin North	9	Awutu Senya East
10	Assin South	10	Gomoa East
11	Agona East		
12	Awutu Senya West		
13	Twifo Heman Lower Denkyira		

APPENDIX 7: List of health facilities (referral facilities)

No.	Forest	No.	Coastal
1	Our Lady of Grace, Breman Asikuma	1	Saltpond Government Hospital, Saltpond
2	Dunkwa Hospital, Dunkwa-On-Offin	2	Winneba Government Hospital, Winneba
3	Praso Hospital, Twifu Praso	3	U.C.C Hospital, Cape Coast
4	Agona Swedru Govermnt Hospital, Swedru	4	Cape Coast Metropolitan Hospital, Bakano
5	Ajumako Hospital, Ajumako	5	Ewim Polyclinic, Ewim
6	St. Francis Xavier Hospital, Fosu	6	Central Regional Hospital, Abura
7		7	Apam Catholic Hospital, Apam
8		8	Ankaful Leprosy/General Hospital, Ankaful
9		9	Ankaful Psychiatric Hospital, Ankaful
10		10	Abura Dunkwa Hospital, Abura Dunkwa
11		11	St. Gregory Catholic Hospital, Budumburam

APPENDIX 5: Ethical clearance

GHANA HEALTH SERVICE ETHICS REVIEW COMMITTEE

In case of reply the number and date of this Letter should be quoted

Our Ref: GHS/RD/ERC/Admin/App/2 of 2020
Your Ref. No.

Research & Development Division
Ghana Health Service
P. O. Box MB 190
Accra.
Digital Address: GA-050-3303
Tel: 233-0302-966628
Fax: 233-0302-683424
Mob: 233-030-3529895
Email: ethics.research@ghanail.org
2nd August, 2020

Mavis Purpelle Dakorah
Department of Biomedical Sciences
University of Cape Coast
Cape Coast

The Ghana Health Service Ethics Review Committee has reviewed and given approval for the implementation of your Study Protocol.

GHS-ERC Number	**GHS-ERC017/03/20**
Study Title	Genetic Characterization of Anti-Malaria Drug Resistant Strains in Central Region of Ghana
Approval Date	2nd August, 2020
Expiry Date	1st August, 2021
GHS-ERC Decision	Approved

This approval requires the following from the Principal Investigator

- Submission of yearly progress report of the study to the Ethics Review Committee (ERC)
- Renewal of ethical approval if the study lasts for more than 12 months,
- Reporting of all serious adverse events related to this study to the ERC within three days verbally and seven days in writing.
- Submission of a final report after completion of the study
- Informing ERC if study cannot be implemented or is discontinued and reasons why
- Informing the ERC and your sponsor (where applicable) before any publication of the research findings.

You are kindly advised to adhere to the national guidelines or protocols on the prevention of COVID-19

Please note that any modification of the study without ERC approval of the amendment is invalid.

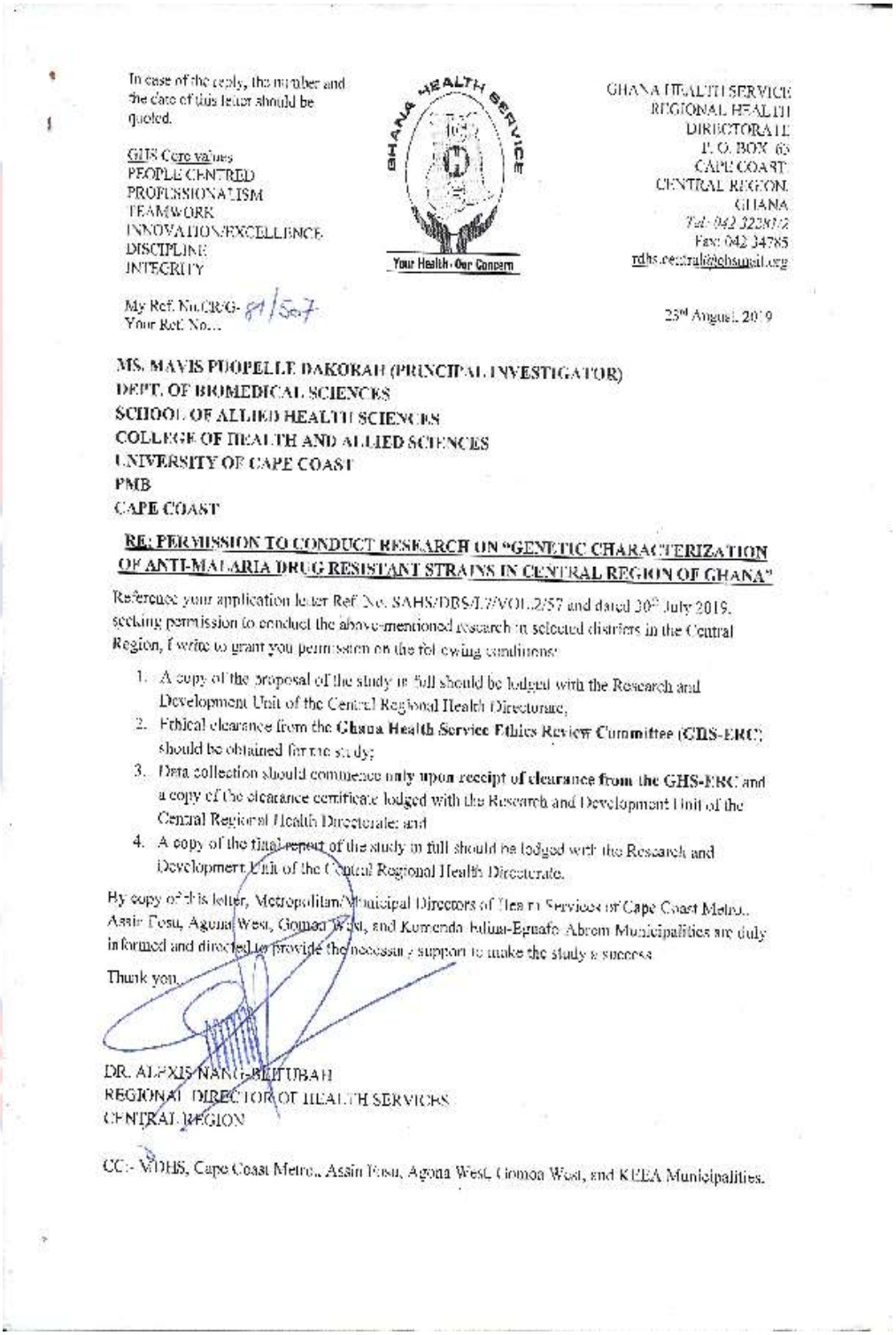
The ERC may observe or ensure to be observed procedures and records of the study during and after implementation.

Kindly quote the protocol identification number in all future correspondence in relation to this approved protocol

SIGNED .....
Dr. Cynthia Baddianaah
(GHS-ERC Chairperson)

Cc: The Director, Research & Development Division, Ghana Health Service, Accra

APPENDIX 6: Letter of approval from the Central Regional health directorate



In case of the reply, the number and the date of this letter should be quoted.

GHS Core values
PEOPLE CENTRED
PROFESSIONALISM
TEAMWORK
INNOVATION/EXCELLENCE
DISCIPLINE
INTEGRITY

GHANA HEALTH SERVICE
REGIONAL HEALTH DIRECTORATE
P. O. BOX 65
CAPE COAST
CENTRAL REGION
GHANA
Tel: 042 32287/2
Fax: 042 34785
rdhs.central@ghsmail.org

My Ref. No.CR/G- 81/507
Your Ref. No...

25th August, 2019

MS. MAVIS PUOPELLE DAKORAH (PRINCIPAL INVESTIGATOR)
DEPT. OF BIOMEDICAL SCIENCES
SCHOOL OF ALLIED HEALTH SCIENCES
COLLEGE OF HEALTH AND ALLIED SCIENCES
UNIVERSITY OF CAPE COAST
PMB
CAPE COAST

RE: PERMISSION TO CONDUCT RESEARCH ON "GENETIC CHARACTERIZATION OF ANTI-MALARIA DRUG RESISTANT STRAINS IN CENTRAL REGION OF GHANA"

Reference your application letter Ref. No. SAHS/DBS/L7/VOL.2/57 and dated 30th July 2019, seeking permission to conduct the above-mentioned research in selected districts in the Central Region, I write to grant you permission on the following conditions:

1. A copy of the proposal of the study in full should be lodged with the Research and Development Unit of the Central Regional Health Directorate;
2. Ethical clearance from the **Ghana Health Service Ethics Review Committee (GHS-ERC)** should be obtained for the study;
3. Data collection should commence **only upon receipt of clearance from the GHS-ERC** and a copy of the clearance certificate lodged with the Research and Development Unit of the Central Regional Health Directorate; and
4. A copy of the final report of the study in full should be lodged with the Research and Development Unit of the Central Regional Health Directorate.

By copy of this letter, Metropolitan/Municipal Directors of Health Services of Cape Coast Metro., Assin Fosu, Agona West, Gomoa West, and Komenda-Edina-Eguafo-Abrem Municipalities are duly informed and directed to provide the necessary support to make the study a success.

Thank you.

DR. ALEXIS NANG-BELTUBAH
REGIONAL DIRECTOR OF HEALTH SERVICES
CENTRAL REGION

CC:- MDHS, Cape Coast Metro., Assin Fosu, Agona West, Gomoa West, and KEEA Municipalities.

APPENDIX 7: Approval Letter from St. Francis Xavier Catholic Hsp.

NATIONAL CATHOLIC HEALTH SERVICES

(ARCHDIOCESE OF CAPE COAST)

Sisters Hospitallers
ST FRANCIS XAVIER HOSPITAL

Our Ref: SFXH/CR/CLIAG/GC/09/299
Your Ref:

14th September, 2020

Ms. Mavis P. Dakorah
Laboratory Department
Cape Coast Teaching Hospital
P. O. Box CT 1363
Cape Coast

Dear Madam,

RE: REQUEST FOR PERMISSION TO CONDUCT RESEARCH IN YOUR FACILITY

I refer to your letter dated 4th March, 2020 requesting for permission to conduct a study on Malaria infected patients which involves **Genetic Characterization of Anti-Malaria Drug Resistant in Central Region.**

Approval is hereby given for you to use St. Francis Xavier Hospital as one of your sites for the exercise.

I count on your assurance of confidentiality of information you would gather. You are also advised to abide by all ethical standards that the facility will orient you on when you report.

Thank you.

Yours faithfully,

SR. GEORGINA DONKING
(SISTER IN CHARGE)

P. O. Box 78, ASSIN FOSU, Ghana (West Africa)
tel: 00233 244 93 43 07
email: sisterhs@sfxhospitallers.org
www.sistershospitallers.org

APPENDIX 8: Approval letter from Abura Dunkwa District Hsp.

In case of the reply the number And the date of this letter should be quoted.

My Ref. No. GHS/ADH/01/2020

Your Ref. No. .....

E-mail:adhosp@yahoo.com

GHANA HEALTH SERVICE

Your Health - Our Concern

DISTRICT HOSPITAL
GHANA HEALTH SERVICE
P. O. BOX 55
ABURA DUNKWA

September 14, 2020

DR. DESMOND O. ACHEAMPONG
UNIVERSITY OF CAPE COAST
COLLEGE OF HEALTH AND ALLIED SCIENCES

RE: REQUEST FOR PERMISSION TO CONDUCT RESEARCH IN YOUR FACILITY
MS. MAVIS P. DAKORAH

Approval has been given to your letter dated 9th March, 2020 requesting to conduct research on Anti-Malaria Drug resistances at Abura Dunkwa Hospital.

Thank you.

DR. TAKYI DUAYEDEN
(AG. MEDICAL SUPT)

APPENDIX 9: Approval Letter from St. Gregory Catholic Hsp.

NATIONAL CATHOLIC HEALTH SERVICE
(Cape Coast Archdiocesan Health Service)

Tel:233-302-936-281
233-541-596-156
Email: st.gregoryh@gmail.com
stgregoryhospital@nchs.org.gh

St. Gregory Catholic Hospital
Gomoa Buduburam
P. O. Box 9 Awutu Bereku

Our ref: SGCH/087/467/11/20
Date: 6th November, 2020

MAVIS P. DAKORAH
DEPT. OF BIOMEDICAL SCIENCE
SCHOOL OF ALLIED HEALTH SCIENCE, UCC
CAPE COAST

Dear Ms. Dakorah,

APPROVAL TO CONDUCT RESEACH ON "GENETIC CHARACTERIZATION OF ANTI-MALARIA DRUG RESISTANAT STRAINS IN CENTRAL REGION OF GHANA"

In reference to you letter dated 9th March, 2020, I write on behalf of management of St. Gregory Catholic Hospital to inform you that your request has been approve.

You are therefore permitted to conduct your research on the topic stated in your letter. Any change in the letter submitted earlier will require a new approval.

Thank you.

Yours faithfully,

Ayegre Mathias Anaba
FDC
(Prn. Health Service Administrator)

IN GOD IS OUR HELP AND HEALTH

APPENDIX 10: Approval letter from Central Regional Health Directorate

In case of the reply the number and the date of this letter should be quoted.

GHS CORE VALUES:

Professionalism
Teamwork
Integrity
Discipline
Excellence
People-Centered

EWIM POLYCLINIC
P. O. BOX K 31
CAPE COAST
CENTRAL REGION
GHANA.
TEL. 03321-33906/96509

5TH OCTOBER, 2020

My Ref. No. GHS/EPC/CC/G-157/144

Your Ref. . . . . .

MS. MAVIS PUOPELLE DAKORAH (PRINCIPAL INVESTIGATOR)
DEPARTMENT OF BIOMEDICAL SCIENCES
SCHOOL OF ALLIED HEALTH SCIENCES
COLLEGE OF HEALTH AND ALLIED SCIENCES
UNIVERSITY OF CAPE COAST
CAPE COAST

RE: REQUEST FOR PERMISSION TO CONDUCT RESEARCH IN YOUR FACILITY

The Regional Director of Health Service letter no. Cr/G-381/507 dated 23rd August, 2019 on the above subject matter in a reply to your earlier application letter Ref. No. SAHS/DDS/17/vol.2/57 dated 30th July, 2019 plus subsequent request letter for permission to conduct research, also dated 9th March, 2020, refers.

Following your successful acquisition of the requisite ethical clearance from the Ghana Health Service Ethics Review Committee, I write to convey management's approval and permission to use Ewim Polyclinic as a preferred selected study site to conduct your earmarked research.

Please, kindly note that this grant of leave is subject to absolute compliance to the dictates of the express conditions of both the Ghana Health Service ethical clearance and the Regional Director of Health Service's authorization.

By a copy of this letter, the head of laboratory, Ewim Polyclinic is directed to provide the researcher with the needed assistance and co-operation.

We wish you well in your endeavor.

Thank you.

CHIEF MEDICAL OFFICER I/C
EWIM POLYCLINIC
P.O. BOX K 31
CAPE COAST

DR. JUSTICE ARTHUR
(AG. MEDICAL SUPERINTENDENT)

APPENDIX 11: Approval to contribute samples to Malaria Genomic Project

Malaria Genome Campus
Wellcome Sanger Institute
Hinxton, Cambridgeshire, United Kingdom, CB10 1SA
22/01/2018

Mr Enoch Aninagyei
PhD Student
Department of Biomedical Sciences
University of Cape Coast
Ghana

Approval as a collaborator to contribute samples to Malaria Genomic Project

I am happy to inform you that the Research Ethics Committee and the Malaria Genomics Research Centre, Hinxton, UK, have granted your request to collaborate with our laboratory.

This approval was granted based on thorough review of your research proposal, material transfer agreement and sample collection tools.

Please note that your collaboration number is 1241-PF-GHANINAGYEI. Please refer to this number in all correspondence with us.

Per this collaboration, you are expected to submit 500 dry blood spots (DBS) for sequencing. Sample collection materials shall, however, be sent to you free of charge.

For any clarifications, do not hesitate to contact Dr Sonia Goncalves on sg1@sagnger.ac.uk.

Thank you very much. We hope to enjoy working with you.

Prof Dominic Kwiatkowski
Director, Malaria Genomic Research Laboratory
Hinxton, UK

APPENDIX 12: Certificate of Accreditation

UNIVERSITY OF CAMBRIDGE

WELLCOME SANGER INSTITUTE
WELLCOME GENOME CAMPUS
HINXTON, CAMBRIDGESHIRE, UNITED KINGDOM
CB10 1SA

CERTIFICATE OF ACCREDITATION

This is to certify that:

Name of Trainee	ID number	Affiliate institution
ANINAGYEI ENOCH	1241-PF-GH-ANINAGYEI	SCHOOL OF ALLIED HEALTH SCIENCES DEPARTMENT OF BIOMEDICAL SCIENCES UNIVERSITY OF CAPE COAST, GHANA

has successfully, undergone intensive theoretical and practical course in genome science and biotechnology. He is accredited as a trainer of trainees to use our training materials to train other scientists on novel Sanger DNA biotechnology and as a referral scientist to interpret and analyse Sanger sequences and biodata in the West Africa sub-region

Prof D. Kwiatkowski
Director
MalariaGen Network

Dated
14-02-2019

Dr Sonia M Goncalves
Project Manager
MalariaGen Network