THE UNIVERSITY OF YAOUNDE I
****
FACULTY OF SCIENCE
****
Centre for Research and Training in Graduate Studies in Life, Health & Environment Sciences

UNIVERSITE DE YAOUNDE I
****
FACULTE DES SCIENCES
****
Centre de Recherche et Formation Doctorale, Science de la Vie, Santé et Environnement

DEPARTMENT OF BIOCHEMISTRY

DEPARTEMENT DE BIOCHIMIE

LABORATORY FOR PUBLIC HEALTH RESEARCH BIOTECHNOLOGIES

LABORATOIRE DE RECHERCHE EN SANTE PUBLIQUE ET BIOTECHNOLOGIES

Pharmacogenomics & Health impact of antimalarial medications in Cameroon and study the efficacy and safety of Sulfadoxine Pyrimethamine and Amodiaquine in the Northern Regions of Cameroon

Thesis presented in partial fulfillment of the requirements for the award of a

Doctorate/PhD in Biochemistry

Speciality: Biotechnology and Development

BIBLIOTHEQUE UNIVERSITAIRE UY1

By

Dr NGONO MBALLA Rose

Matricule: 89H736

PharmD. (Aix Marseille, 1988) / 3rd Cycle Doctorate (UY1, 1993)

Supervisor

MBACHAM FON Wilfred

Professor

University of Yaoundé 1

Year 2020

UNIVERSITE DE YAOUNDE I
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FACULTE DES SCIENCES
****
CENTRE DE RECHERCHE ET DE FORMATION DOCTORALE EN SCIENCES DE LA VIE-SANTE ET ENVIRONNEMENT
****
UNITE DE RECHERCHE ET DE FORMATION DOCTORALE EN SCIENCES DE LA VIE
****

DEPARTEMENT DE BIOCHIMIE

THE UNIVERSITY OF YAOUNDE I
****

FACULTY OF SCIENCES
****
CENTRE FOR RESEARCH AND TRAINING SCHOOL FOR LIFE SCIENCE HEALTH AND ENVIRONMENT
****
RESEARCH AND DOCTORATE TRAINING UNIT OF LIFE SCIENCE
****

DEPARTMENT OF BIOCHEMISTRY

ATTESTATION DE CORRECTION DU MEMOIRE

Nous soussignons: Prof Rose Leke, Président du jury et Prof Stephen Ghogomu, examinateur, attestons que Mme **Ngono Mballa Rose (Matricule-89H736)** a effectué les corrections conformément aux exigences du jury de soutenance de son mémoire de Doctorat/PhD en Biochimie option-Biotechnologie et Developpement sur le thème : **"Pharmacogenomics & Health Impact of Antimalaria Medication in Cameroon and a Case Study of the Efficacy and Safety of Sulphadoxine/Pyrimethamine-Amodiaquine (SPAQ) in the Northern Regions of Cameroon".** En date du 11 Aout, 2021.

En foi de quoi la présente attestation lui est établie et délivrée pour servir et valoir ce que de droit.

Examinateur

Stephen M. Ghogomu

Président du jury

Pr. Rose Leke
Présidente du Conseil d'Administration
de l'IMPM

Chef de Département

23 SEPT 2021

Professor
Enzymology-Toxicology

UNIVERSITE DE YAOUNDE I
UNIVERSITY OF YAOUNDE I
Département de Biochimie
Department of Biochemistry
Le Chef de Département
The Head of Department
FACULTY OF SCIENCE
FACULTE DE SCIENCES

LIST OF PERMENENT TEACHING STAFF

THE UNIVERSITY OF YAOUNDE I **FACULTY OF SCIENCE** Division of Programming and follow-up of Academic Affaires		**UNIVERSITE DE YAOUNDE I** **FACULTE DES SCIENCES** Division de la Programmation et du Suivi des Activités Académiques
LIST OF PERMENENT TEACHING STAFF		**LISTE DES ENSEIGNANTS PERMANENTS**

ADMINISTRATION

DEAN : TCHOUANKEU, *Professor*

VICE-DEAN / DPSAA : DONGO Etienne, *Professor*

VICE-DEAN / DSSE : AJEAGAH Gidéon AGHAINDUM

VICE-DEAN / DRC : ABOSSOLO Monique, Associate Professor

Chief Administrative and Financial Division: NDOYE FOE Marie C. F., *Associate Professor*

Chief Division of Research, Academic and Students Affairs, MBAZE MEVA'A Luc Léonard, *Associate Professor*

Department of Biochemistry (BC) (40)			
N°	**First Name and Names**	**Grade**	**Observations**
1	BENG née NINTCHOM PENLAP V.	Professor	Present
2	FEKAM BOYOM Fabrice	Professor	Present
3	MBACHAM FON Wilfred	Professor	Present
4	MOUNDIPA FEWOU Paul	Professor	Head of Department
5	OBEN Julius ENYONG	Professor	Vice-Dean/DSSE
6	ATOGHO TIEDEU Barbara Mma	Associate Professor	Present
7	BELINGA née NDOYE FOE Marie C. F.	Associate Professor	Chef DAF / FS
8	BIGOGA JUDE	Professor	Present
9	BOUDJEKO Thaddée	Associate Professor	Present
10	EFFA ONOMO Pierre	Associate Professor	Present
11	FOKOU Elie	Professor	Present
12	KANSCI Germain	Professor	Present
13	NGUEFACK Julienne	Associate Professor	Present
14	NGONDI Judith Laure	Associate Professor	Present
15	NJAYOU Frédéric Nico	Associate Professor	Present
16	WAKAM née NANA Louise	Associate Professor	Present
17	ACHU Merci BIH	Associate Professor	Present
18	AKINDEH MBUH NJI	Senior Lecturer	Present
19	AZANTSA Boris	Associate Professor	Present
20	DEMMANO Gustave	Senior Lecturer	Present
21	DJOKAM TAMO Rosine	Senior Lecturer	Present
22	DJUIDJE NGOUNOUE Marcelline	Senior Lecturer	Present
23	DJUIKWO NKONGA Ruth Viviane	Senior Lecturer	Present
24	EWANE Cécile Anne	Senior Lecturer	Present
25	KOTUE TAPTUE Charles	Senior Lecturer	Present
26	LUNGA Paul KAILAH	Senior Lecturer	Present
27	MASUMBE NETONGO Palmer	Senior Lecturer	Present
28	MBONG A. MOUGANDE Mary Ann	Senior Lecturer	Present
29	MOFOR née TEUGWA Clautilde	Associate Professor	IA4/MINESUP
30	PECHANGOU NSANGOU Sylvain	Senior Lecturer	Present
31	TCHANA KOUATCHOUA Angèle	Associate Professor	Present
32	BEBOY EDJENGUELE Sara Nathalie	Senior Lecturer	Present
33	DAKOLE DABOY Charles	Senior Lecturer	Present
34	DONGMO LEKAGNE Joseph Blaise	Senior Lecturer	Present
35	FONKOUA Martin	Senior Lecturer	Present
36	MANANGA Marlyse Joséphine	Senior Lecturer	Present
37	BEBEE FADIMATOU	Assistant Lecturer	Present
38	MBOUCHE FANMOE Marcelline Joëlle	Assistant Lecturer	Present

Department of Biology and Animal physiology (B.P.A.) (44)

1	BILONG BILONG Charles Félix	Professor	Head of Department
2	DIMO Théophile	Professor	Present
3	FOMENA Abraham	Professor	Present
4	KAMTCHOUING Pierre	Professor	Present
5	MIMPFOUNDI REMY	Professor	Present
6	NJAMEN Dieudonné	Professor	Present
7	NJIOKOU Flobert	Professor	Present
8	NOLA Moïse	Professor	Present
9	TAN Paul	Professor	Present
10	TCHUEM TCHUENTE Louis	Professor	Coord. Progr. MINSANTE
11	AJEAGAH Gidéon AGHAINDOUM	Associate Professor	Chef Serv. /Diplomation FS
12	DJIETO Lordon Champlain	Associate Professor	Present
13	DZEUFIET DJOMENI Paul Désiré	Associate Professor	Present
14	ESSOMBA née NTSAMA MBALLA	Associate Professor	VDRC/Chef dépt FMSB
15	FOTO MENBOHAN Samuel	Associate Professor	CT2 MIN. ENERGIE
16	KAMGANG René	Associate Professor	C.E. MINRESI
17	KEKEUNOU Sévilor	Associate Professor	Present
18	MEGNEKOU Rosette	Associate Professor	Present
19	MONY NTONE Ruth	Associate Professor	Present
20	ZEBAZE TOGOUET Serge Hubert	Associate Professor	Present
21	ALENE Désirée Chantal	Senior Lecturer	Chef Serv. /MINSUP
22	ATSAMO Albert Donatien	Senior Lecturer	Present
23	BELLET EDIMO Oscar Roger	Senior Lecturer	Present
24	BILANDA Danielle Claude	Senior Lecturer	Present
25	DJIOGUE Séfirin	Senior Lecturer	Present
26	GOUNOUE KAMKUMO Raceline	Senior Lecturer	Present
27	JATSA MEGAPTCHE Hermine	Senior Lecturer	Present
28	LEKEUFACK FOLEFACK Guy Benoît	Senior Lecturer	Present
29	MAHOB Raymond Joseph	Senior Lecturer	Present
30	MBENOUN MASSE Paul Serge	Senior Lecturer	Present
31	MOUGANG NGAMENI Luciane	Senior Lecturer	Present
32	NGOUATEU KENFACK Omer BEBE	Senior Lecturer	Present
33	NGUEGUIM TSOFACK Florence	Senior Lecturer	Present
34	NGUEMBOCK	Senior Lecturer	Present
35	NJUA Clarisse YAFI	Senior Lecturer	Present
36	TADU Zéphirin	Senior Lecturer	Present
37	TOMBI Jeannette	Senior Lecturer	Present
38	YEDE	Senior Lecturer	Present
39	ETEME ENAMA Serge	Assistant Lecturer	Present
40	KANDEDA KAVAYE Antoine	Assistant Lecturer	Present
41	KOGA MANG'Dobara	Assistant Lecturer	Present

42	MECHI DONGFACK Mireille Flore	Assistant Lecturer	Present
43	NOAH E. O. Vivien	Assistant Lecturer	Present
44	MVEYO NDANKEU Yves Patrick	Assistant Lecturer	Present
Department of Biology and Plant physiology (B.P.V.) (27)			
1	YOUMBI Emmanuel	Professor	Head of Department
2	AMBANG Zachée	Professor	DAARS / UYII
3	MOSSEBO Dominique Claude	Professor	Present
4	BELL Joseph Martin	Associate Professor	Present
5	DJOCGOUE Pierre François	Associate Professor	Present
6	KENGNE NOUMSI Ives Magloire	Associate Professor	Present
7	MBOLO Marie	Associate Professor	Coordo. Programme MINFOF
8	NDONGO BEKOLO	Associate Professor	CE / MINRESI
9	ZAPFACK Louis	Associate Professor	Present
10	ANGONI Hyacinthe	Senior Lecturer	Present
11	BIYE Elvire Hortense	Senior Lecturer	Present
12	MAHBOU SOMO TOUKAM Gabriel	Senior Lecturer	Present
13	MALLA Armand William	Senior Lecturer	Present
14	MBARGA BINDZI Marie Alain.	Senior Lecturer	Inspecteur académ. N°1 MINESUP
15	NGALLE Hermine BILLE	Senior Lecturer	Present
16	NGODO MELINGUI Jean Baptiste	Senior Lecturer	Present
17	NGONKEU MAGAPTCHE Eddy L.	Senior Lecturer	Present
18	NGOUO Lucas Vincent	Senior Lecturer	Present
19	NSOM ZAMO Annie Claude ép. Pial	Senior Lecturer	Expert national./UNESCO
20	ONANA Jean Michel	Senior Lecturer	Present
21	TONFACK Libert Brice	Senior Lecturer	Present
22	TSOATA Esaïe	Senior Lecturer	Present
23	DJEUANI Astride Carole	Assistant Lecturer	Present
24	GONMADGE Christelle	Assistant Lecturer	Present
25	MAFFO MAFFO Nicole Liliane	Assistant Lecturer	Present
26	NNANGA MEBENGA Ruth Laure	Assistant Lecturer	Present
27	NOUKEU KOUAKAM Armelle	Assistant Lecturer	Present
Department of Inorganic Chemistry (C.I.) (35)			
1	AGWARA ONDOH Moïse	Professor	Vice-Recteur/ Ubda
2	KETCHA MBADCAM Joseph	Professor	Head of Department
3	NDIFON Peter TEKE	Professor	CT MINRESI
4	NGAMENI Emmanuel	Professor	Dean/ UD
5	GHOGOMU Paul MINGO	Professor	Minister in charge of Mission, PRC
6	LAMINSI Samuel	Professor	Present
7	MELO née CHINJE Uphie F.	Professor	Recteur/UN
8	NDIFON Peter TEKE	Professor	CT MINRESI

9	NGAMENI Emmanuel	Professor	Dean/ UDS
10	BABALE née DJAM DOUDOU	Associate Professor	Present
11	DJOUFAC WOUMFO Emmanuel	Associate Professor	Present
12	ELIMBI Antoine	Associate Professor	Present
13	KONG SAKEO	Associate Professor	Present
14	NDIKONTAR Maurice KOR	Associate Professor	Vice-Dean/Ubda
15	NGOMO Horace MANGA	Associate Professor	Recteur /UB
16	YOUNANG Elie	Associate Professor	Present
17	ACAYANKA Elie	Senior Lecturer	Present
18	CHEUMANI YONA Arnaud	Senior Lecturer	Present
19	EMADACK Alphonse	Senior Lecturer	Present
20	GWET Simon – Pierre	Senior Lecturer	Present
21	KAMGANG YOUBI Georges	Senior Lecturer	Present
22	KEUMEGNE MBOUGUEM Jean Claude	Senior Lecturer	Present
23	KENNE DEDZO Gustave	Senior Lecturer	Present
24	MBEY Jean Aimé	Senior Lecturer	Present
25	NDI Julius NSAMI	Senior Lecturer	Present
26	NDOSIRI Bridget NDOYE	Senior Lecturer	Present
27	NJIOMOU C. épse DJANGANG	Senior Lecturer	Present
28	NJOYA Dayirou	Senior Lecturer	Present
29	NYAMEN Linda Dyorisse	Senior Lecturer	Present
30	PABOUDAM GBAMBIE Awaou	Senior Lecturer	Present
31	TCHAKOUTE KOUAMO Hervé	Senior Lecturer	Present
32	BELIBI BELIBI Placide Désiré	Assistant Lecturer	Present
33	KOUOTOU DAOUDA	Assistant Lecturer	Present
34	MAKON Thomas Beauregar	Assistant Lecturer	Present
35	NCHIMI NONO Katia	Assistant Lecturer	Present
Department of Organic Chemistry (C.O.) (33)			
1	DONGO Etienne	Professor	Vice-Dean/DPSAA
2	GHOGOMU TIH ROBERT RALPH	Professor	Directeur I.B.A. Foumban
3	MBAFOR Joseph	Professor	Present
4	NGADJUI TCHALEU B.	Professor	Head of dpt FMBS
5	NGOUELA Silvère Augustin	Professor	Head of dpt Uds
6	NKENGFACK Augustin Ephraïm	Professor	Head of Department
7	NYASSE Barthélemy	Professor	Directeur /UN
8	PEGNYEMB Dieudonné Emmanuel	Professor	Directeur au MINESUP
9	WANDJI Jean	Professor	Present
10	Alex de Théodore ATCHADE	Associate Professor	DEPE/ UYI
11	FOLEFOC Gabriel NGOSONG	Associate Professor	Present
12	KEUMEDJIO Félix	Associate Professor	Present
13	KOUAM Jacques	Associate Professor	Present
14	MBAZOA née DJAMA Céline	Associate Professor	Present
15	NOUNGOUE TCHAMO Diderot	Associate Professor	Present
16	TCHOUANKEU Jean-Claude	Associate Professor	Vice-Recteur/ UYII

17	TIH née NGO BILONG E. Anastasie	Associate Professor	Present
18	YANKEP Emmanuel	Associate Professor	Present
19	TCHUENDEM KENMOGNE Marguerite	Associate Professor	Present
20	AMBASSA Pantaleon	Senior Lecturer	Present
21	EYONG Kenneth OBEN	Senior Lecturer	Present
22	FOTSO WABO Ghislain	Senior Lecturer	Present
23	KAMTO Eutrophe Ledoux	Senior Lecturer	Present
24	MKOUNGA Pierre	Senior Lecturer	Present
25	NGO MBING Joséphine	Senior Lecturer	Present
26	NGONO BIKOBO Dominique Serge	Senior Lecturer	Head of Cell/ MINRESI
27	NOTE LOUGBOT Olivier	Senior Lecturer	CEA/MINESUP
28	OUAHOUO WACHE Blandine Marlyse	Senior Lecturer	Present
29	TABOPDA KUATE Turibio	Senior Lecturer	Present
30	TAGATSING FOTSING Maurice	Senior Lecturer	Present
31	ZONDEGOUMBA Ernestine	Senior Lecturer	Present
32	NGINTEDO Dominique	Assistant Lecturer	Present
33	NGOMO Orléans	Assistant Lecturer	Present
Department of Informatic (IN) (26)			
1	FOUDA NDJODO Marcel	Professor	IGA-MINESUP/Chef Dpt ENS
2	ATSA ETOUNDI Roger	Professor	Head of Department Head of Division MINFOPRA
3	NDOUNDAM René	Associate Professor	Present
4	CHEDOM FOTSO Donatien	Senior Lecturer	Present
5	DJAM Xaviera Youth KIMBI	Senior Lecturer	Present
6	KOUOKAM KOUOKAM Etienne A.	Senior Lecturer	Present
7	MELATAGIA YONTA Paulin	Senior Lecturer	Present
8	MOTO MPONG Serge Alain	Senior Lecturer	Present
9	TINDO Gilbert	Senior Lecturer	Present
10	TSOPZE Norbert	Senior Lecturer	Present
11	WAKU KOUAMOU Jules	Senior Lecturer	Present
12	ABESSOLO ALO'O Gislain	Assistant Lecturer	Present
13	AMINOU Halilou	Assistant Lecturer	Present
14	BAYEM Jacques Narcisse	Assistant Lecturer	Present
15	DOMGA KOMGUEM Rodrigue	Assistant Lecturer	Present
16	EBELE Serge	Assistant Lecturer	Present
17	HAMZA Adamou	Assistant Lecturer	Present
18	JIOMEKONG AZANZI Fidel	Assistant Lecturer	Present
19	KAMDEM KENGNE Christiane	Assistant Lecturer	Present
20	KAMGUEU Patrick Olivier	Assistant Lecturer	Present
21	MAKEMBE S. Fritz Oswald	Assistant Lecturer	Present
22	MEYEMDOU Nadège Sylvianne	Assistant Lecturer	Present
23	MONTHE DJIADEU Valery Martial	Assistant Lecturer	Present
24	NKONDOCK MI BAHANACK Nicolas	Assistant Lecturer	Present
25	OLE OLE David Claude	Assistant Lecturer	Present

26	TAPAMO KENFACK Hyppolite	Assistant Lecturer	Present
Department of Mathamatics (MA) (30)			
1	BEKOLLE David	Professor	Vice-Recteur UN
2	BITJONG NDOMBOL	Professor	Present
3	DOSSA COSSY Marcel	Professor	Present
4	AYISSI Raoult Domingo	Associate Professor	Head of Department
5	EMVUDU WONO Yves S.	Associate Professor	Directeur MINESUP
6	NKUIMI JUGNIA Célestin	Associate Professor	Present
7	NOUNDJEU Pierre	Associate Professor	Present
8	AGHOUKENG JIOFACK Jean Gérard	Senior Lecturer	C.E. MINEPAT
9	BOGSO Antoine M	Senior Lecturer	Present
10	CHENDJOU Gilbert	Senior Lecturer	Present
11	DOUANLA YONTA Hermann	Senior Lecturer	Present
12	FOMEKONG Christophe	Senior Lecturer	Present
13	KIANPI Maurice	Senior Lecturer	Present
14	KIKI Maxime Armand	Senior Lecturer	Present
15	MBAKOP Guy Merlin	Senior Lecturer	Present
16	MBANG Joseph	Senior Lecturer	Present
17	MBEHOU Mohamed	Senior Lecturer	Present
18	MBELE BEDIMA Martin	Senior Lecturer	Present
19	MENGUE MENGUE David Joe	Senior Lecturer	Present
20	NGUEFACK Bertrand	Senior Lecturer	Present
21	POLA DOUNDOU Emmanuel	Senior Lecturer	Present
22	TAKAM SOH Patrice	Senior Lecturer	Present
23	TCHANGANG Roger Duclos	Senior Lecturer	Present
24	TCHOUNDJA Edgar Landry	Senior Lecturer	Present
25	TIAYA TSAGUE N. Anne- Marie	Senior Lecturer	Present
26	DJIADEU NGAHA Michel	Assistant Lecturer	Present
27	MBIAKOP Hilaire George	Assistant Lecturer	Present
28	NIMPA PEFOUKEU Romain	Assistant Lecturer	Present
29	TANG AHANDA Barnabé	Assistant Lecturer	Chef Serv. MINPLAMAT
30	TETSADJIO TCHILEPECK Mesmin E.	Assistant Lecturer	Present
Department of Microbiology (MB) (13)			
1	ETOA François-Xavier	Professor	Head of Department,
2	ESSIA NGANG Jean Justin	Professor	Chef de Div recherche IMPM
3	BOYOMO ONANA	Associate Professor	Present
4	NWAGA Dieudonné M.	Associate Professor	Present
5	NYEGUE Maximilienne Ascension	Associate Professor	Present
6	RIWOM Sara Honorine	Associate Professor	Present
7	SADO KAMDEM Sylvain Leroy	Associate Professor	Present
8	BODA Maurice	Senior Lecturer	Present
9	BOUGNOM Blaise Pascal	Senior Lecturer	Present

10	ENO Anna Arey	Senior Lecturer	Present
11	ESSONO OBOUGOU Germain G.	Senior Lecturer	Present
12	NJIKI BIKOÏ Jacky	Assistant Lecturer	Present
13	TCHIKOUA Roger	Assistant Lecturer	Present
Department of Physics (PH) (41)			
1	ESSIMBI ZOBO Bernard	Professor	Present
2	KOFANE Timoléon Crépin	Professor	Present
3	NDJAKA Jean Marie Bienvenu	Professor	Present
4	NJOMO Donatien	Professor	Present
5	PEMHA Elkana	Professor	Present
6	TABOD Charles TABOD	Professor	Dean/Ubda
7	TCHAWOUA Clément	Professor	Present
8	WOAFO Paul	Professor	Present
9	BEN- BOLIE Germain Hubert	Associate Professor	Present
10	BIYA MOTTO Frédéric	Associate Professor	Dir.Gén. B. MEKIM
11	DJUIDJE KENMOE G. épse ALOYEM K.	Associate Professor	Present
12	EKOBENA FOUDA Henri Paul	Associate Professor	Chef Dépt UN
13	EYEBE FOUDA Jean Sire	Associate Professor	Present
14	FEWO Serge Ibraïd	Associate Professor	Present
15	MBANE BIOUELE	Associate Professor	Present
16	NANA NBENDJO Blaise	Associate Professor	Present
17	NJANDJOCK NOUCK Philippe	Associate Professor	Chef Serv. MINRESI
18	NOUAYOU Robert	Associate Professor	Present
19	OUMAROU BOUBA	Associate Professor	Recteur UY II
20	SIEWE SIEWE Martin	Associate Professor	Present
21	ZEKENG Serge Sylvain	Associate Professor	Present
22	BODO Bernard	Senior Lecturer	Present
23	EDONGUE HERVAIS	Senior Lecturer	Present
24	EYEBE FOUDA Jean Sire	Senior Lecturer	Present
25	ENYEGUE A NYAM F. épse BELINGA	Senior Lecturer	Present
26	FOUEDJIO David	Senior Lecturer	Present
27	HONA Jacques	Senior Lecturer	Present
28	MBINACK Clément	Senior Lecturer	Present
29	MBONO SAMBA Yves Christian U.	Senior Lecturer	Present
30	NDOP Joseph	Senior Lecturer	Present
31	OBOUNOU Marcel	Senior Lecturer	Present
32	SIMO Elie	Senior Lecturer	Present
33	TABI Conrad Bertrand	Senior Lecturer	Present
34	TCHOFFO Fidèle	Senior Lecturer	Present
35	VONDOU DERBETINI Appolinaire	Senior Lecturer	Present
36	WAKATA née BEYA Annie	Senior Lecturer	Chef Serv. MINESUP
37	WOULACHE Rosalie Laure	Senior Lecturer	Present
38	ABDOURAHIMI	Assistant Lecturer	Present
39	CHAMANI Roméo	Assistant Lecturer	Present

40	MELI'I Jorelle Larissa	Assistant Lecturer	Present
41	MVOGO Alain	Assistant Lecturer	Present
Department of Heath Sciences (S.T.) (42)			
1	NDJIGUI Paul-Désiré	Professor	Head of Department
2	NZENTI Jean-Paul	Professor	Present
3	BITOM Dieudonné Lucien	Professor	Dean FASA/ U D
4	ABOSSOLO née ANGUE Monique	Associate Professor	Chef DAASR FS/UYI
5	FOUATEU Rose épouse YONGUE	Associate Professor	Present
6	KAMGANG Pierre	Associate Professor	Present
7	MEDJO EKO Robert	Associate Professor	Conseiller Technique/UYII
8	MOUNDI Amidou	Associate Professor	CT/MINMIDT
9	NDAM NGOUPAYOU Jules-Remy	Associate Professor	Present
10	NGOS III Simon	Associate Professor	D.A.A.C./UM
11	NJILAH Isaac KONFOR	Associate Professor	Present
12	NKOUMBOU Charles	Associate Professor	Present
13	TEMDJIM Robert	Associate Professor	Present
14	YENE ATANGANA Joseph Q.	Associate Professor	Chef de Divi/ MINTP
15	BEKOA Etienne	Senior Lecturer	Present
16	BISSO Dieudonné	Senior Lecturer	Directeur Pjt. B. Memve'ele
17	EKOMANE Emile	Senior Lecturer	Present
18	ESSONO Jean	Senior Lecturer	Present
19	FUH Calistus Gentry	Senior Lecturer	Serv D'ETAT /MINMIDT
20	GANNO Sylvestre	Senior Lecturer	Present
21	GHOGOMU Richard TANWI	Senior Lecturer	Chef de Dépt IMIP/Maroua
22	LAMILEN BILLA Daniel	Senior Lecturer	Present
23	MBIDA YEM	Senior Lecturer	Present
24	MINYEM Dieudonné	Senior Lecturer	Chef de Dépt IMIP/Maroua
25	MOUAFO Lucas	Senior Lecturer	Present
26	NGO BELNOUN Rose Noël	Senior Lecturer	Present
27	NGO BIDJECK Louise Marie	Senior Lecturer	Present
28	NGUEUTCHOUA Gabriel	Senior Lecturer	CEA MINRESI
29	NJOM Bernard de Lattre	Senior Lecturer	Present
30	NYECK Bruno	Senior Lecturer	Present
31	ONANA Vincent	Senior Lecturer	Present
32	TCHAKOUNTE J. épse. NUMBEM	Senior Lecturer	CEA MINRESI
33	TCHOUANKOUE Jean-Pierre	Senior Lecturer	Present
34	ZO'O ZAME Philémon	Senior Lecturer	DG ART
35	TEMGA Jean Pierre	Senior Lecturer	Present
36	ANABA ONANA Achille Basile	Assistant Lecturer	Present
37	BINELI BETSI Thierry Olivier	Assistant Lecturer	Present
38	METANG Victor	Assistant Lecturer	Present
39	NOMO NEGUE Emmanuel	Assistant Lecturer	Present
40	SABABA Elisé	Assistant Lecturer	Present
41	TCHAPTCHET T. De Pesquidoux I	Assistant Lecturer	Present

42	TEHNA Nathanaël	Assistant Lecturer	Present

Repartition by Department

Department	Number				
	Pr	**AP**	**SL**	**AL**	**Total**
BCh	5 (1)	10 (5)	17 (9)	08 (4)	**40 (19)**
BPA	10 (0)	10 (2)	18 (7)	6 (2)	**44 (11)**
BPV	03 (0)	06 (1)	13 (3)	05 (5)	**27 (9)**
C.I.	09 (1)	07 (1)	15 (4)	4 (1)	**35 (7)**
C.O.	09 (0)	10 (2)	12 (3)	2 (0)	**33 (5)**
IN	02 (0)	01 (0)	08 (0)	15 (3)	**26 (3)**
MA	03 (0)	04 (0)	18 (1)	05 (0)	**30 (1)**
MB	01 (0)	06 (2)	04 (1)	02 (1)	**13 (4)**
PH	08 (0)	13 (1)	16 (3)	04 (1)	**41 (5)**
ST	03 (0)	10 (1)	22 (4)	07 (0)	**42 (5)**
Total	**53 (2)**	**78 (15)**	**143 (35)**	**58 (17)**	**331 (69)**

Total : **331 (69)** dont

- Professors 53 (2)
- Associate Professors 78 (15)
- Senior Lecturers 143 (35)
- Assistant Lecturers 58 (17)

( ) = Number of Women

The Dean of the Faculty of Science

DEDICATION

The Broad YETENGA Family Group.

Children:: Karen, Antoine Junior, Marina, Lucrèce and Brice Joel

Grand Children: Jade, Grâce and Gedeon Enzo

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List of Abbreviations

ACPR	**:**	Adequate clinical and parasitological response
ACT	**:**	Artemisinin-based Combination Therapy
AE	**:**	Adverse Events
AIDS	**:**	Acquired Immune Deficiency Syndrome
ANSM	**:**	French National Agency for the Safety of Medicines
API	**:**	Active pharmaceutical ingredient
BamH1	**:**	Bacillus amyloliquefaciens Type II
BPM	**:**	Beats per minute.
CBC	**:**	Cameroon Baptist Convention Health Board
CDC	**:**	Centers for Disease Control and Prevention
Cgmp	**:**	Cyclic Guanosine Monophosphate
CFRs	**:**	Case Report Forms
CTD	**:**	Common Technical Document
CYP	**:**	Cytochrome
DEET	**:**	Diethylmetatoluamide
DHA	**:**	Dihydroartemisinin
DHA-P	**:**	DHA-Piperaquine
DHFR	**:**	Dihydrofolate Reductase
DHPS	**:**	Dihydropteroate Synthase
DNA	**:**	Deoxyribonucleic acid
dNTPs	**:**	Deoxynucléotide Triphosphate
DP	**:**	Dihydroartemisinin-piperaquine
DRA	**:**	Drug Regulatory Authority
dTTP	**:**	Desoxythymidine Triphosphate
EDTA	**:**	Ethylene diamine tetra acetic acid
eGMP	**:**	e-Good Manufacturing Pratices
ELISA	**:**	Enzyme-linked Immunosorbent Assay
ETF	**:**	Early treatment failure
FDA	**:**	Food and Drug Administration
FOLH1	**:**	Folate Hydrolase I
G6PD	**:**	Glucose 6 Phospho Dehydrogenase
GMP	**:**	Good Manufacturing Practice

HOAT	**:**	Hepatic Human Organic Anion Transporter
HPLC	**:**	High Pressure Liquid Chromatography
HPPK	**:**	Hydroxymethylpterin Pyrophosphokinase
IPT	**:**	Intermittent Preventive Treatment
IPTc	**:**	Intermittent Preventive Treatment in children
IPTi	**:**	Intermittent Preventive Treatment in infants
IPTp	**:**	Intermittent Preventive Treatment in pregnancy
IRS	**:**	Indoor Residual Spraying
ITMs	**:**	Improved traditional medicines
ITNs	**:**	Insecticide Treated Nets
Kpn1	**:**	Restriction Enzyme
LANACOME:		National Drug Quality Control and Valuation Laboratory
LBW	**:**	Low Birth Weight
LCF	**:**	Late clinical failure
LLINs	**:**	Long Lasting Insecticide-treated Nets
LPF	**:**	Late parasitological failure
MDR	**:**	Multidrug Resistance
MHRA	**:**	Medicine and Healthcare Regulatory Agency
Mmhg	**:**	Millimeters of mercury
MMV	**:**	Malaria Medicines Venture
MSP	**:**	Merozoite Surface Protein
MTHFR	**:**	Methylenetetrahydropholate reductase
NAFDAC	**:**	National Agency for Food and Drug Administration and Control
NAT 2	**:**	N-acetyl transferase 2
NDRA	**:**	National Drug Regulatory Authority
NMCP	**:**	National Malaria Control Programme
OCT1	**:**	Organic Cation Transporter 1
PBS	**:**	Phosphate buffer saline
Pfcrt	**:**	Plasmodium falciparum chloroquine resistance transporter
Pfdhfr	**:**	Plasmodium Falciprum dihydrofolate redustase
***Pf*NHE 1**	**:**	Plasmodium Falciprum Na+/H+ Exchanger **1**
Pfmdr-1	**:**	Plasmodium Falciparum Multi Drug Resistance 1
Pfmsp-1	**:**	Plasmodium Falciparum Merozoite Surface Protein 1
pLDH	**:**	Parasite Lactate Dehydrogenase.

PCR	**:**	Polymerase Chain Reaction
PNLP	**:**	Programme National de Lutte Contre le Paludisme
pRBC	**:**	parasitized Red Blood Cells
PVC	**:**	Polyvinyl Chloride
QBC	**:**	Quantitative buffy coat
QMS	**:**	Quality Management System
RFLP	**:**	Restriction Fragment Length Polymorphism
RDT	**:**	Rapid Diagnostic Test
SAE	**:**	Severe Adverse Events
SERCA	**:**	Sarco/endoplasmic reticulum Ca^{2+} ATPase
SFM	**:**	Substandard and Falsified Medicines
SMC	**:**	Seasonal Malaria Chemoprevention
SNP	**:**	Single Nucleotide Polymorphisms
SP	**:**	Sulphadoxine-pyrimethamine
SPAQ	**:**	Sulfadoxine-Pyrimethamine+Amodiaquine
TBE	**:**	Tris Boric-acid EDTA
TMP	**:**	Thymidine-5'-monophosphate
TLC	**:**	Thin Layer Chromatography
TS	**:**	Thymidylate Synthetase
USP	**:**	United States Pharmacopoea
WHO	**:**	World Health Organization

ABSTRACT

Malaria remains a real public health problem in the world despite all the efforts and the means implemented to overcome it. The poor quality and fake antimalarials, constitute an important health and economic problem.The present study aims to analyze the situation of the irrational use of antimalarials in Cameroon after a systematic review of published studies; the physico chemical analysis of Artesunate and Amodiaquine done in combination with pharmaco-technical tests, as well as the study of the effectiveness of sulfadoxine-pyrimethamine-amodiaquine (SP+AQ), versus artésunate+amodiaquine (AS+AQ), in a 2-arm, randomized, controlled study; it was also about to determine the pharmacogenomic profile (using the polymorphisms of the Cytochrome P2C8 (CYP2C8) and N-Acetyl Transferase 2 (NAT2) genes) in children aged 6 months to 10 years, with acute uncomplicated *P. falciparum* malaria, from the North and Far North regions of Cameroon. The DNA of Plasmodium falciparum was extracted by the Chelex method. The merozoite surface protein 2 (msp2) gene was amplified in the confirmed treatment failure cases, in order to differentiate between recrudescence and re-infection. Molecular markers for Sulfadoxine+Pyrimethamine drug resistance used, were genetic polymorphisms in the dihydrofolate réductase (dhfr) gene. Genotyping was done by polymerase chain reaction – restriction fragment length polymorphism (PCR-RFLP); the portions containing the C59R and S108N polymorphisms of the dhfr gene, the CYP2C8 gene, and the NAT2 gene were amplified by PCR, followed by enzymatic digestion with specific enzymes (*XmnI+BsrI*); *BclI* and (*BamH1+Kpn1+Taq1*) respectively. Finally, software STRING and MEGA were used to determine protein-protein interactions from the N Acetyl transferase 2 (NAT2) protein and the others. All the 15 batches of (AS+AQ) screened were under-dosed, non-compliant, largely imported from India and found mainly in hospitals and health centers. A total of 235 children, treated for the trial with (AS+AQ)) and (SP+AQ) were followed up for 28 days to assess the response to treatment. Early treatment failure (ETF) cases recorded were 24 (20.5%) in the AS+AQ group and 21(17.8%) in the SP+AQ group. Adequate clinical and parasitological response (ACPR) cases observed on day 14 were 99 (84.6%) in the AS+AQ group and 91 (77.1%) in the SP+AQ group. ACPR cases observed on day 28 were 61 (71.8%) in the AS+AQ group and 59 (7 3.75%) in the SP+AQ group. The cure rates were respectively at 91.8% and 91.4% in the AS+AQ and in SP+AQ. However, recrudescence was observed in 7 cases, with respectively 3 and 4cases in the AS+AQ and in the SP+AQ group. After digestion of the dhfr gene in recrudescence cases, the C59R mutation was observed in all, while the S108N mutation was observed in 5 ones. The pharmagenomy profile allowed to identify, two alleles: CYP2C8*1 (76%) and CYP2C8*2; we found 70% fast metabolizers phenotypes for the CYP2C8 gene. The NAT2*6 allele was higher (39%) than the NAT2*5/6 genotype (28.12%). After treatment, 63.5% presented an adequate clinical and parasitological response (ACPR) and 24% an early treatment failure (ETF). In the regions of North Cameroon, the wild CYP2C8 allele and the associated rapid metabolizers, as well as the NAT2*5/6 genotype were more present; the fast phenotype was much more susceptible to experience early treatment failures and much more vulnerable to experience late parasitological failures.

Key words: Malaria, Artesunate+Amodiaquine, fake medicines, Cytochrome P2C8, N-acetyl transferase 2, metabolyzers, treatment failure, sub-standard anti-malarials.

RESUME

Le paludisme reste un véritable problème de santé publique dans le monde malgré tous les efforts et les moyens déployés pour le vaincre. Les médicaments de mauvaise qualité et les faux médicaments, en particulier les antipaludéens, constituent un problème sanitaire et économique important. La présente étude vise à analyser l'utilisation irrationnelle des antipaludiques au Cameroun après une revue bibliographique,à étudier l'efficacité de la (SP+AQ) versus (AS+AQ), dans un essai clinique randomisé et contrôlé à 2 bras ; il a été aussi question de déterminer le profil pharmacogénomique, à partir des polymorphismes des gènes cytochrome P2C8 (CYP2C8) et N-Acetyl transférase 2 (NAT2) chez les enfants âgés de 6 mois à 10 ans, atteints de paludisme simple à *Plasmodium falciparum*, et vivant dans les régions du Nord et de l'Extrême du Nord du Cameroun. L'ADN du parasite a été extrait par la méthode de Chelex. Le gène de la protéine[2] de surface du mérozoite (*msp*2) a été amplifié pour différencier les recrudescences des réinfections. Le génotypage a été fait par la **réaction en chaine par polymérase** du Polymorphisme des longueurs de fragments de restriction (PCR-RFLP) ; les portions contenant les polymorphismes aux sites C59R et S108N du gène dihydrofolate reductase (*dhfr),* le gène *CYP2C8*, et le gène NAT2 ont été amplifiés par la PCR, suivi de la digestion enzymatique avec les enzymes (*XmnI+BsrI) ; BclI et (BamH1+Kpn1+Taq1)* respectivement. Enfin, les logiciels STRING et MEGA ont été utilisés pour déterminer les interactions protéines-protéines à partir de la protéine *NAT2* et les autres. Tous les 15 lots d' (AS+AQ) analysés étaient sous-dosés, non conformes, en grande partie importés d'Inde et majoritairement trouvés dans les hôpitaux et les centres de santé. Les deux cent trente cinq enfants traités durant l'essai avec les combinaisons (AS+AQ)) et (SP+AQ) on été suivis pendant 28 jours pour évaluer leur réponse au traitement. Les cas d'échec précoce au traitement enregistrés étaient 24 (20,5 %) dans le groupe AS+AQ et 21 (17.8%) dans le groupe SP+AQ. La réponse clinique et parasitologique adéquate observée au jour 14 était 99 (84,6 %) dans le groupe AS+AQ et 91 (77,1 %) dans le groupe SP+AQ. La réponse clinique et parasitologique adéquate observée sur 28 jours était de 61 (71,8 %) dans le groupe AS+AQ et 59 (73.75 %) dans le groupe SP+AQ. Le taux de guérison dans le groupe AS+AQ était de 91,8 % tandis que le taux de guérison de SP+AQ était de 91,4 %. Cependant, la recrudescence a été observée dans 7 cas, avec respectivement 3 et 4 cas dans les groupes (AS+AQ) et (SP+AQ). Après la digestion du gène *dhfr* dans les cas de recrudescence, la mutation de C59R a été observée dans tous ces cas, tandis que la mutation de S108N a été observée chez 5 des cas de recrudescence. Le profil pharmacogénomique a révélé deux allèles : prédominance de l'allèle sauvage CYP2C8*1 (76%), et CYP2C8*2 ; on a trouvé 70% de phénotypes métaboliseurs rapides pour le gene CYP2C8. La fréquence de l'allèle NAT2*6 était plus élevée (39%) que le génotype NAT2*5/6 (28,12%). Après le traitement, 63,5% présentait une réponse clinique et parasitologique adéquate (ACPR) et 24% d'échec précoce de traitement (ETF).Dans les régions du nord du Cameroun, l'allèle sauvage CYP2C8 et les métaboliseurs rapides associés ainsi que le génotype NAT2*5/6 étaient plus présents ; le phénotype rapide était beaucoup plus susceptible de donner des échecs précoces de traitement et plus vulnérables à des échecs parasitologiques tardifs.

Mots-clés: Malaria, Artesunate Amodiaquine, Cytochrome P2C8, N-acetyl transferase 2, metaboliseurs, Echecs thérapeutiques, antipaludiques.

GENERAL INTRODUCTION

INTRODUCTION

Malaria infection is one of the major causes of deaths in the African continent. The disease is caused by blood infection of protozoan parasites of the genus Plasmodium, which is transmitted from one human to another by infected female Anopheles mosquitoes. Five Plasmodium species routinely infect humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium knowlesi* (Singh *et al.,* 2004). The high burden of malaria in Africa is due to *P. falciparum*, which adapts and co-specializes with Anopheles gambiae, the most effective and widespread malaria vector. Since 2000, the incidence of malaria has been reduced by 17% and malaria mortality rates by 26%. However, the rate of decline has stalled and even reversed in some regions since 2014. In 2018 as described by the latest World malaria report, 228 million malaria cases were reported worldwide, compared with 251 million cases in 2010 and 231 million cases in 2017 in 91 countries, and the global tally of malaria deaths reached 405 000 deaths, compared with 416 000 estimated deaths in 2017. Despite these achievements, the African region continues to account for about 93% of malaria cases and deaths worldwide (WHO, 2018).

As indicated by the National SWWProgram for Cameroon (PNLP), malaria is still the major burden of public health and represent more than 24% of all consultations and 46% of all hospitalizations with 25.9% of malaria related deaths in children below 5 years in Cameroon (PNLP, 2018). The unprecedented malaria outbreak in October 2013 in Maroua (Far North Region of Cameroon) which left over 12,000 people hospitalised and at least 600 dead, placed the efficacy of the antimalarial drugs in that region into question. It is important to note that more than 3,500 of the recorded cases were children. Between 1986 and 1992, antimalarial drug studies assessing the efficacy of drugs in Cameroon demonstrated various levels of resistance to Chloroquine varying between 40-86% and 20-25% in the South and North Regions respectively, with an inverse low Mefloquine resistance in the South (2%) but high Mefloquine resistance in the North (25%) (Brasseur *et al.,* 1986). An *in vivo* study in Bangangte (West Cameroon) in 1995 revealed that 50% of *P. falciparum* malaria patients were resistant to chloroquine and 26.3% to Amodiaquine (Brasseur *et al.,* 1986). The major factors contributing to the increase of this resistance of the *Plasmodium falciparum* to antimalarial drugs are the improper use of antimalarial drugs, for long periods, resulting in the appearance of the phenomenon of resistance making these treatments ineffective (Songue *et al.*, 2013), the substandard/ falsified/ counterfeit and irrational use of anti-malarial drugs.

The Ministry of Public Health decided based on the resolutions of a technical expert group meeting (WHO, 2011), to implement the Seasonal Malaria Chemoprevention in the Sahel sub-region in 2013. Trials of SulfadoxinePyrimethamine (SP) in combination with Amodiaquine in children below 5 years, that have demonstrated safety and high efficacy in Mali, Central African Republic and Cameroon (Maiga *et al.,* 2015; Mbacham *et al.,* 2010 ; Nambei *et al.,* 2013 ; Tekete *et al.,* 2011). Furthermore, with the recent malaria upsurge in Maroua which took the lives of more than 1000 individuals (mostly women and children), there is the need to further understand the treatment responses to antimalarial combination therapies for children in this part of the country. The initiation of Seasonal Malaria Chemoprevention in the northern regions of Cameroon could prevent such outbreaks from reoccurring. To speed up the implementation of Seasonal Malaria Chemoprevention in Cameroon, after evaluation and situational analysis of irrational use of antimalarials and the presence of fake medicines circulating in the country a clinical trial to assess the efficacy of SP+AQ in the North and Far North Regions of Cameroon was therefore conducted with AS+AQ being the comparator drug, after the outbreak in Maroua, which touched mostly children of schooling age. The merozoite surface protein (*msp2*) gene was used in the study to determine recrudescence from reinfected cases. We found that the most frequent mutations in the *dhfr* gene are C59R and S108N, while studied in the recrudescent cases to determine SP resistance in the study. Added to this, polymorphisms in *CYP2C8* and *NAT2* genes that may help in prescription of drugs with regard to the genetic status of each patient may influence treatment outcomes among individuals suffering from uncomplicated malaria were also studied. To contribute to this knowledge in Cameroon, the present study was carried out to: i)Master the situational analysis of irrational use of antimalarials and the extend of the presence of fake medicines circulating in the country; ii) Distinguish the single nucleotide polymorphisms (SNPs) of CYP2C8, NAT2 (NAT2*5, NAT2*6 and NAT2*7) and to decipher their relationship with the treatment results; including the quality of some antimalaria ASAQ and the impact regarding pharmacoeconomics issuses; iii) Study Pharmacogenomic profiles of the population of the Northern Regions, including their cladogram.

HYPOTHESES AND OBJECTIVES

HYPOTHESES

1. There is not situational consequence of the irrational use of antimalarial in Cameroon,
2. Sulfadoxine-Pyrimethamine+Amodiaquine, as the Principal Protocol for Seasonal Malaria Chemoprevention, has no effect on treatment efficacy in children between 6 months and 10 years of age in the Northern Regions of Cameroon,
3. There are no variations in the genes of drug metabolism that may influence treatment outcomes and safety among individuals suffering from uncomplicated malaria in the Northern Regions of Cameroon.

OBJECTIVES

Main objective

The main objective of this study was to assess the role of pharmacogenomic factors in treatment outcomes among individuals with uncomplicated malaria, pharmacology and health impact of some antimalarial medicines though in the Northern Regions of Cameroon.

Specific objectives

1. To analyze the situation of the irrational use, the presence and extent of substandard/ falsified/ counterfeit antimalaria drugs in Cameroon.
2. To investigate the antimalaria efficacy of SPAQ versus ASAQ in the Northern Regions, with emphasis on clinical trials analysis and treatment outcomes.
3. To determine the pharmacogenomic profile of trial patients from Northern Regions of Cameroon by using CYP2C8 and NAT2 gene polyorphisms involved in the antimalaria drug metabolism, including their impact in treatment outcomes.

CHAPTER ONE :

LITERATURE REVIEW

BACKGROUND AND JUSTIFICATION

Malaria infection is one of the most devastating parasitic diseases of humans and one of the major causes of deaths in the African continent, especially in Sub-Saharan Africa. The high burden of malaria in Africa is due to *P. falciparum*, whose vector is Anopheles gambiae, the most effective and widespread malaria vector. Since 2000, the incidence of malaria has been reduced by 17% and malaria mortality rates by 26%. However, the rate of decline has stalled and even reversed in some regions since 2014. Despite these achievements, the African region continues to account for about 92% of malaria cases and deaths worldwide. Despite the numerous control efforts against malaria, Cameroon, like many other African countries, following the increasing resistance of chloroquine (Mbacham *et al.,* 2005) had adopted Amodiaquine and Sulphadoxine-pyrimethamine as first and second- line drugs in 2002 and 2004, respectively. Unfortunately, the cure rate of these two drugs was proven to deteriorate as monotherapies in five study sites of Cameroon (Mbacham *et al.,* 2005).

A number of studies were carried out between 1986 and 1992 when monotherapies were still the drugs for treatment of malaria in Cameroon to assess their efficacy. These investigations have reported different levels of resistance in different regions. According to the studies, depended on what anti-malarial drug was being considered; chloroquine resistance was shown to vary between 40-86% in the south and 20-25% in the north. Mefloquine resistance was rather found to be lower in the south (2%) than in the north (25%) (Brasseur *et al.,* 1986; Brasseur *et al.,* 1988; Brasseur *et al.,* 1992; Brasseur *et al.,* 1992a; Brasseur *et al.,* 1992b). In 1995, this resistance was also found in west region (at Bangante) where 50% of patients with *P. falciparum* malaria were resistant to to chloroquine and 26.3% to Amodiaquine (Agnamey *et al.,* 1995). A further rise in antifolate clinical failures of about 48% was observed in Limbe and Nkambe (Mbacham *et al.,* 2005). Recent studies demonstrated Amodiaquine failure rates higher than 15-20% in Mali, Nigeria and Cameroon (Happi *et al.,* 2006; Mbacham *et al.,* 2010; Tekete *et al.,* 2009; Tekete *et al.,* 2011).

The main drugs recommended to stop the spread of resistance were mainly Artemisinin-based combinations (short-acting drugs) with long-acting drugs such as Lumefantrine, Piperaquine and SP. ASAQ which is the first line of treatment of acute uncomplicated *P. falciparum* malaria in Cameroon (PNLP, 2014). Unfortunately, most ASAQs are developed in Asia, introduced into the African market without a rigorous drug development standards and an

appropriate post-market surveillance system. Based on this, it is therefore important for us to track these substandard/falsified/counterfeit antimalaria drugs and study their efficacy to better contribute to the elimination of this disease.

I.1. Malaria, epidemiology and case incidence rate

The global incidence rate (number of cases per 1000 population) of malaria reduced between 2010 and 2018; it fell from 71 in 2010 to 57 in 2018. However, from 2014 to 2018, the rate of change slowed dramatically, reducing from 60 in 2013 to 57 in 2014 and remaining at similar levels through to 2018. In 2018, an estimated 228 million cases of malaria occurred worldwide, compared with 251 million cases in 2010 and 231 million cases in 2017. The estimated number of malaria deaths stood at 405 000 in 2018, compared with 416 000 deaths in 2017. Most malaria cases in 2018 were in the World Health Organization (WHO) African Region (213 million or 93%), followed by the WHO South-East Asia Region with 3.4% of the cases and the WHO Eastern Mediterranean Region with 2.1%. Nineteen countries in sub-Saharan Africa and India carried almost 85% of the global malaria burden (WHO, 2018).

The highest reductions in incidence were seen in the WHO South-East Asia Region, mainly owing to reductions in India, Indonesia and countries in the Greater Mekong subregion. The geographic distribution of malaria case incidence by country is shown in Fig. 1 (WHO, 2018).

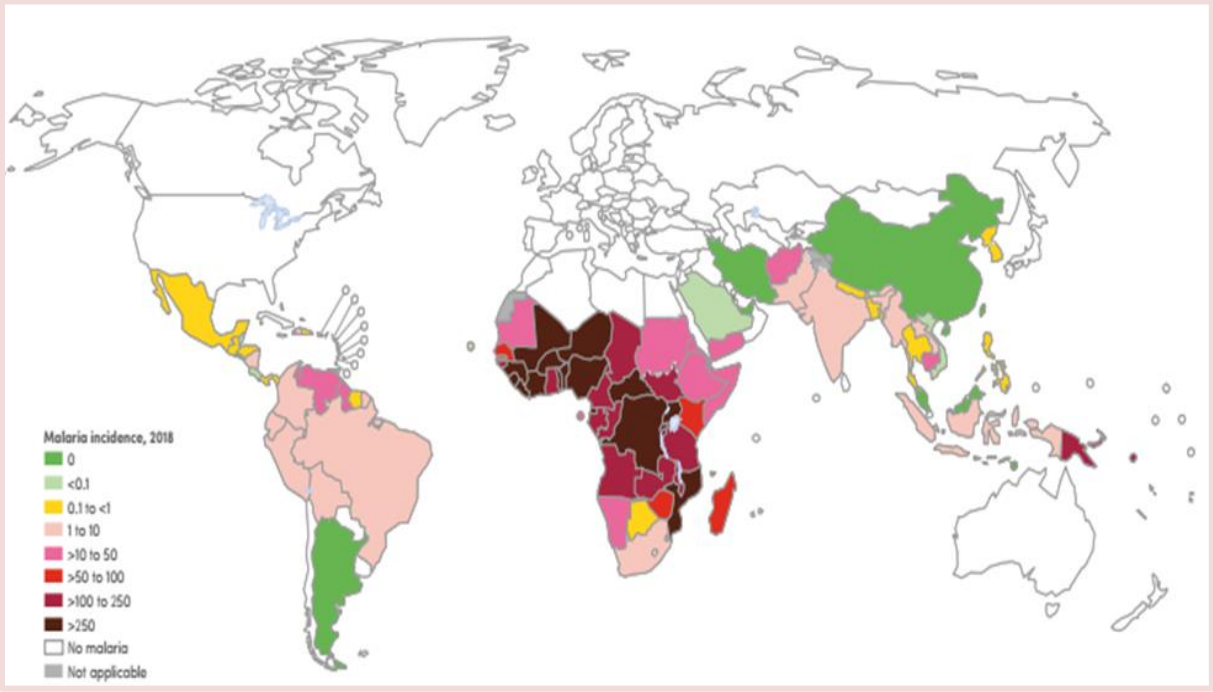


Figure 1: Map of malaria case incidence rate (*cases per 1000 population at risk*) by country, 2018 Source: *WHO estimates*

I.2. Malaria diagnosis

Direct microscopic examination of intracellular parasites on stained blood films is the current standard for definitive diagnosis in nearly all settings. However, several other approaches exist or are in development.

I.2.1. Microscopy

Simple light microscopic examination of Giemsa stained blood films is the most widely practised and useful method for definitive malaria diagnosis. Advantages include differentiation between species, quantification of the parasite density, and ability to distinguish clinically important asexual parasite stages from gametocytes which may persist without causing symptoms. These advantages can be critical for proper case-management and evaluating parasitological response to treatment. Specific disadvantages are that slide collection, staining, and reading can be time-consuming and microscopists need to be trained and supervised to ensure consistent reliability. While availability of microscopic diagnosis has been shown to reduce drug use in some trial settings (Jonkman *et al.*, 1995).

I.2.2. Clinical (presumptive)

Although reliable diagnosis cannot be made on the basis of signs and symptoms alone because of the non-specific nature of clinical malaria, clinical diagnosis of malaria is common in many malarious areas. In much of the malaria-endemic world, resources and trained health personnel are so scarce that presumptive clinical diagnosis is the only realistic option. Clinical diagnosis offers the advantages of ease, speed, and low cost. In areas where malaria is prevalent, clinical diagnosis usually results in all patients with fever and no apparent other cause being treated for malaria. This approach can identify most patients who truly need antimalarial treatment, but it is also likely to misclassify many who do not (Olivar *et al.*, 1991). Over-diagnosis can be considerable and contributes to misuse of antimalarial drugs.

Considerable overlap exists between the signs and symptoms of malaria and other frequent diseases, especially acute lower respiratory tract infection (ALRI), and can greatly increase the frequency of misdiagnosis and mistreatment (Redd *et al.*, 1992). The Integrated Management of Childhood Illnesses (IMCI) programme defined an algorithm that has been developed in order to improve diagnosis and treatment of the most common childhood illnesses in areas relying upon relatively unskilled health care workers working without access

to laboratories or special equipment. With this algorithm, every febrile child living in a "high-risk" area for malaria should be considered to have, and be treated for, malaria. "High risk" has been defined in IMCI Adaptation Guides as being any situation where as little as 5% of febrile children between the ages of 2 and 59 months are parasitaemic (WHO, 1997), a definition that will likely lead to significant over-diagnosis of malaria in areas with low to moderate malaria transmission.

I.2.3. Antigen detection tests (also known as rapid or "dipstick" tests)

A third diagnostic approach involves the rapid detection of parasite antigens using rapid immunochromatographic techniques. Multiple experimental tests have been developed targeting a variety of parasite antigens (Khusmith *et al.,* 1987). A number of commercially available kits are based on the detection of the histidine-rich protein 2 (HRP-II) of *P. falciparum*. Compared with light microscopy and QBC, this test yielded rapid and highly sensitive diagnosis of *P. falciparum* infection (WHO, 1996). Advantages to this technology are that no special equipment is required, minimal training is needed, the test and reagents are stable at ambient temperatures, and no electricity is needed. The principal disadvantages are a currently high per-test cost and an inability to quantify the density of infection. Furthermore, for tests based on HRP-II, detectable antigen can persist for days after adequate treatment and cure ; therefore, the test cannot adequately distinguish a resolving infection from treatment failure due to drug resistance, especially early after treatment (WHO, 1996).

I.2.4. Molecular tests

Detection of parasite genetic material through polymerase-chain reaction (PCR) techniques is becoming a more frequently used tool in the diagnosis of malaria, as well as the diagnosis and surveillance of drug resistance in malaria. Specific primers have been developed for each of the four species of human malaria. One important use of this new technology is in detecting mixed infections or differentiating between infecting species when microscopic examination is inconclusive (Beck, 1999). In addition, improved PCR techniques could prove useful for conducting molecular epidemiological investigations of malaria clusters or epidemics (Freeman *et al.,* 1999). Primary disadvantages to these methods are overall high cost, high degree of training required, need for special equipment, absolute requirement for electricity, and potential for cross-contamination between samples.

I.3. Cameroon, Geographic location and situation of Malaria

I.3.1. Geographic location of Cameroon

Cameroon is situated in central Africa, within the Gulf of Guinea at a latitude between 2–13°N and a longitude between 9–16°E. It has a surface area of approximately 475,000 km2 with a population of about 24 million (Figure 2). It is bordered to the West by Nigeria, to the North and East by Chad, to the East by Central African Republic and to the South by Congo, Gabon and Equatorial Guinea (BUCREP, 2010).

The country also has a coastal border of about 400 km with the Atlantic Ocean. Data from the demographic and health survey (DHS) and from the malaria indicator survey (MIS), indicated vegetation and altitude as important predictors of the geographical distribution of malaria in Cameroon (WHO, 2015). During the last decade an increase in temperature of 0.4°C and decrease in rainfall of 10–20% have been reported, compared to the period 1951–1980 (Sighomnou, 2004). Across sub-Saharan Africa, similar projections have been reported with an increase in temperature of 1.5 °C above the 1951–1980 baseline level (Serdeczny *et al.*, 2016).

I.3.2. Situation of Malaria in Cameroon

In Cameroon, all the ten regions are affected by malaria. There are at least 48 Anopheles species that have been reported in the country, with 14 implicated in human malaria transmission. The most common Anopheles species found in the country are: An gambiae, An. arabiensis, An. funestus, An. moucheti and An. nili. Although more than 200 Plasmodium species are said to cause malaria in vertebrates, four main species are known to infect humans: P. falciparum, P. vivax, P. ovale and P. malariae. P. falciparum is the most common predominant species in the country, followed by P. malariae, P. ovale. and P. knowlesi. P. falciparum is found in the tropics and sub-tropics around the world and is responsible for the vast majority of severe forms of clinical disease and all malaria related deaths in Africa. P. vivax only enters red cells that contain the Duffy blood group. Depending on the geo-ecological environment and the duration of intensity of transmission, Cameroon is partitioned into 5 transmission zones: sahelian zone, soudanian zone, sahelo-Guinean zone, Humid Savannah, forest zone (Antonio-Nkondjio *et al.,* 2019).

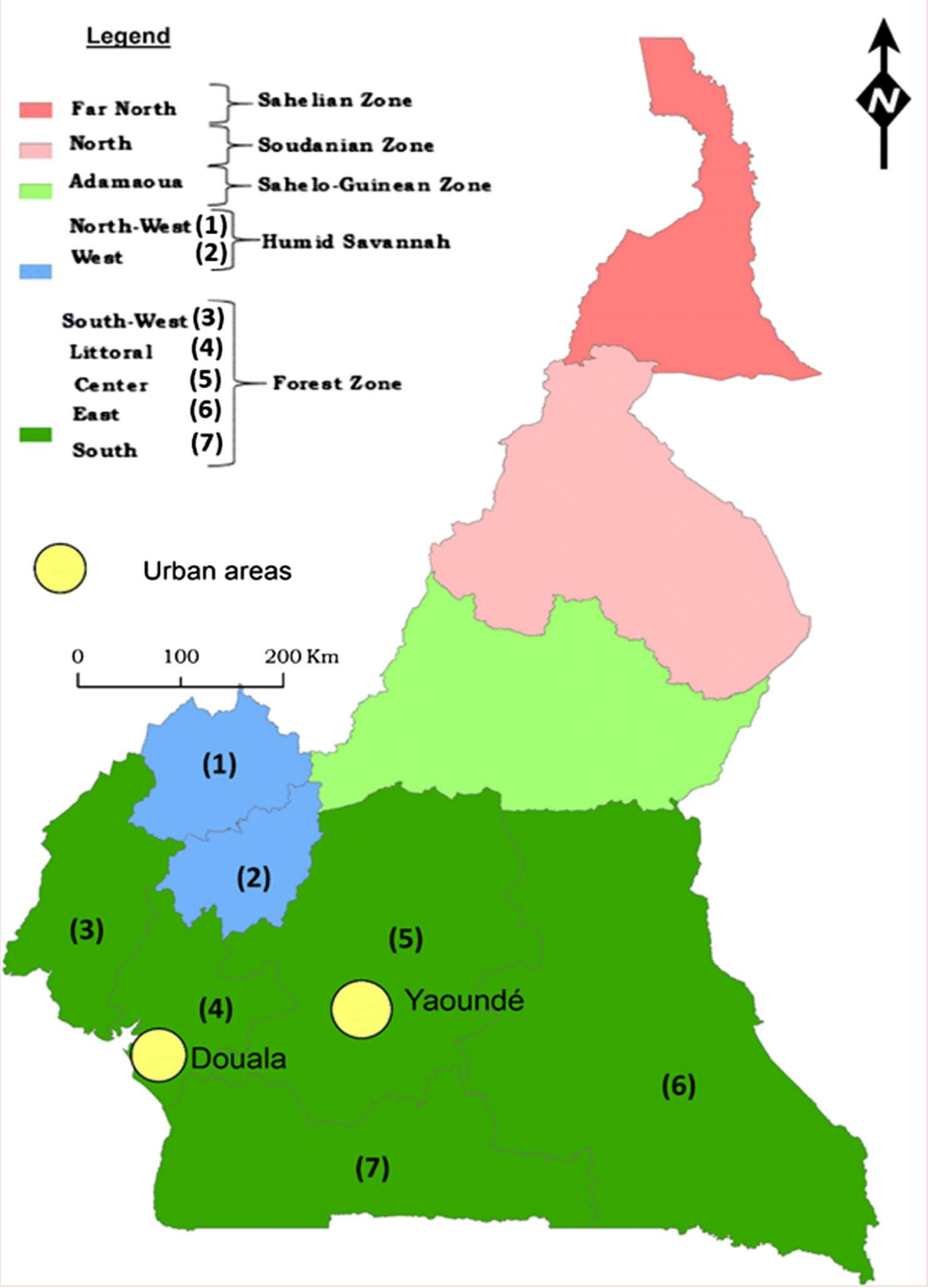


Figure 2: Malaria transmission zones in Cameroon, climatic and administrative divisions (Antonio-Nkondjio et al., 2019).

I.4. Life cycle of *Plasmodium sp* and mode of transmission

I.4.1. *Plasmodium falciparum* life cycle

The life cycle of the parasite is complex and takes place successively in humans (asexual phase in the intermediate host) and in the Anopheles (sexual stage in the definitive host) (figure 3).

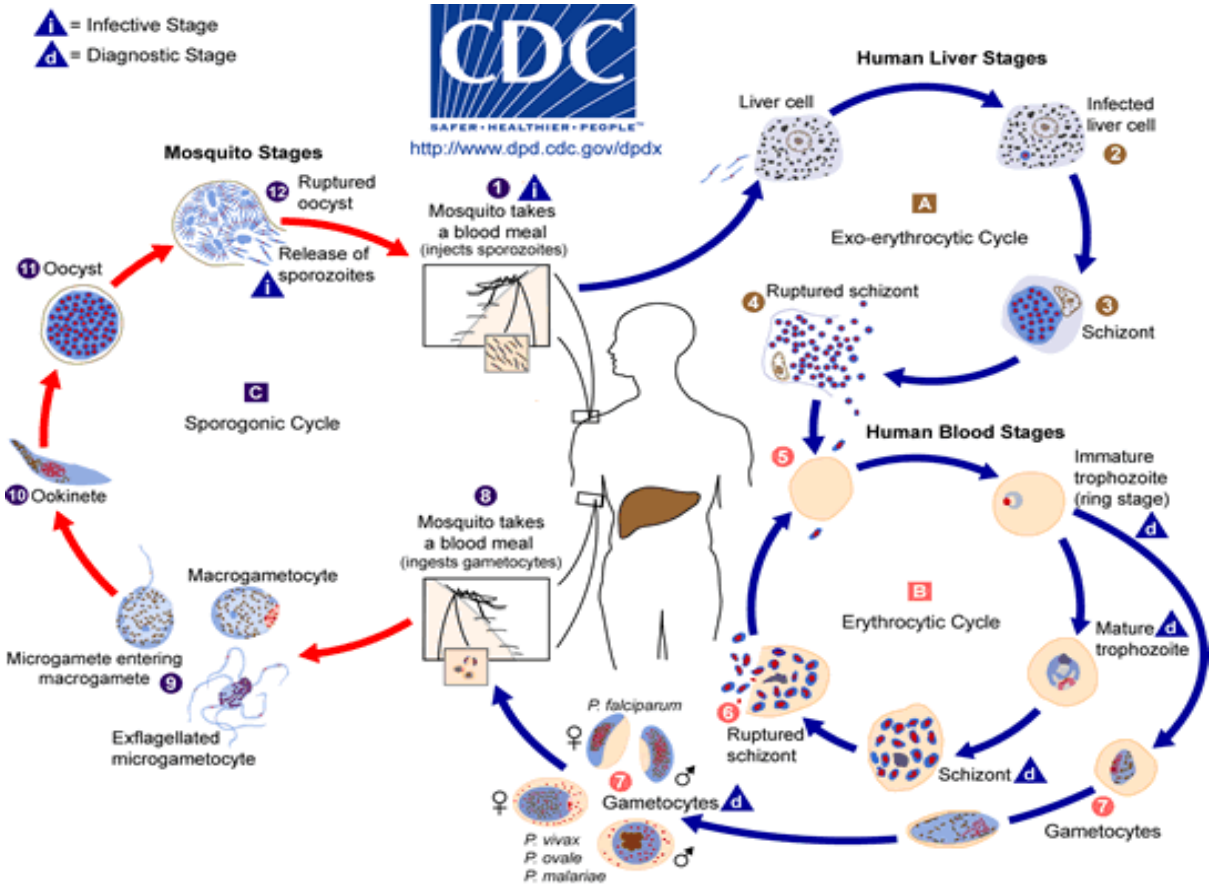


Figure 3: Plasmodium falciparum life cycle

(Source: http://www.dpd.cdc.gov/dpdx/HTML/Malaria.htm)

Anopheles mosquito inoculates sporozoites into the human host 1. *Sporozoites infect liver cells* 2 *and mature into schizonts* 3, *which rupture and release merozoites* 4. *After this initial replication in the liver (exo-erythrocytic schizogony* A*), the parasites undergo asexual multiplication in the erythrocytes (erythrocytic schizogony* B*). Merozoites infect red blood cells* 5. *Trophozoites mature into schizonts, which rupture releasing merozoites* 6. *Some differentiate into sexual stages (gametocytes)* 7. *Gametocytes ingested by Anopheles mosquito during a blood meal* 8. *Parasites' multiplication in the mosquito is the sporogonic cycle* C. *In the mosquito's stomach, microgametes penetrate the macrogametes generating zygotes* 9. *Zygotes become motile and elongated (ookinetes)* 10 *which invade the midgut of the mosquito and develop into oocysts* 11. *Oocysts grow, rupture, and release*

sporozoites, which migrate to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host perpetuates the malaria life cycle.

Pre-erythrocytic stage (liver stage)

With the infested female Anopheles mosquito bite, invasive sporozoites are inoculated into the bloodstream. The number of sporozoites per bite varies from dozens to hundreds. The sporozoites are cleared from the circulation and invade hepatocytes (Soulard *et al.*, 2015). The liver sporozoites initially appear as a mononucleated round body in the cytoplasm of the host cell, where it subsequently begins to develop and multiply asexually to produce a ultinucleate schizont. The cytoplasm of the schizont then separates around individual nuclei to form mononuclear merozoites. A single sporozoite of P. falciparum can result in the replication of 30,000 merozoites (fewer in other species). The length of the schizogonic liver cycle, also called the pre-patent period varies with species and in P. falciparum it is typically about 6 days. P. vivax and P. ovale sporozoites may remain dormant in the liver. Termed as hypnozoites, they may develop into schizonts after a few weeks to months prior to primary infection and cause relapses. Despite this relatively high number of sporozoites that leave the skin and move to the liver, the capacity of each of these sporozoites to result in asexual erythrocytic-stage infections is low. In humans, it takes the bites of five P. falciparum-infected mosquitoes to ensure that 100% of volunteers become infected (Carosi & Castelli, 1997).

Erythrocytic stage (blood stage)

Merozoites released from the hepatic schizonts invade red blood cells a few minutes following release and grow into trophozoites. A vacuole is produced by the parasite which assumes the characteristic ring form. Hemoglobin is ingested into parasite cytoplasmic vacuoles where it is degraded, detoxified and sequestrated as hemozoin (malaria pigment). The trophozoite undergoes a series of asexual division to form a multi nucleate schizont, and matures as the cytoplasm fragments into mononuclear merozoites within the red cell forming a segmenter. The mature schizont (segmenter) then ruptures, releasing daughter merozoites (36 for P. falciparum, 24 for *P. vivax and P. ovale,* and 12 for *P. malariae*) which attach to and enter new red cells, hence, repeating the blood stage schizogonic cycle until the process is inhibited by the specific immune response or by chemotherapy. This cycle takes around 48 hours in P. falciparum. Continuous invasion of red cells by rapidly exponential growing merozoites eventually leads to the appearance of the characteristic symptoms of malaria,

dominated by fever which occurs every 48 hours for *P. falciparum, P. vivax and P. ovale*, and every 72 hours for P. malariae. They are thereby named tertian and quartan fevers respectively (Carosi & Castelli, 1997). A small proportion of merozoites enlarge without nuclear division resulting into large mononuclear parasites within the red cells called gametocytes. P. falciparum gametocytes develop after several repeated erythrocytic cycles, in contrast to *P. vivax, P. ovale* and *P. malariae,* whose gametocytes appear early in infection. Gametocytes are essential in transmitting the infection to others but cause no disease (Cowman & Crabb, 2006).

Sexual reproduction (sporogony in mosquitoes)

Female Anopheles mosquitoes become infected when they ingest blood from an infected human. The male gametocyte's (microgametocyte) nucleus divides into 8 sperm-like flagellated nuclei that are liberated from the red cell as microgametes. This process is called exflagellation. The female gametocyte (macrogametocyte) on the other hand sheds from the red cell and remains free in the extracellular space as a single macrogamete. Fusion occurs, forming an immobile diploid zygote within the gut of the mosquito. The zygote develops into an elongated motile ookinete, which actively penetrates the peritrophic membrane and epithelium of the midgut. It then settles beneath the basal lamina of the outer gut wall where it develops into a non-motile oocyst. The oocyst replicates asexually on several counts (sporogony) resulting in the formation of an average of 10,000 sporozoites, though the number of sporozoites produced in one oocyst varies according to the parasite species. The sporozoites move to the haemocelomic space of the insect and migrate to the salivary glands of the mosquito. Here they are very motile, more infectious and immunogenic than the oocyst sporozoites. The lifecycle restarts when the sporozoite-infected mosquito bites an individual during a blood meal (Carosi & Castelli, 1997).

I.4.2. Mode of transmission

The malaria parasite can be transmitted through several ways which include; transfer of parasitized red cells from an infected mother to the child transplacentally or during labor in which case it is known as congenital malaria (Serra-Casas et al., 2011) during transfusion of blood from infected donors, or through needle-stick injuries (Kitchen and Chiodini, 2006) often accidentally among health care professionals or due to needle sharing among drug addicts. In addition to these, the malaria parasite is principally transmitted by the bite of an

infected female Anopheles mosquito. Sporozoites contained in the saliva of the mosquito (vector) are inoculated into the blood of a human host when the mosquito takes a blood meal. Once in the human host, the parasite continues part of its life cycle which had started in the mosquito host.

I.5. Genetic Diversity of *plasmodium falciparum*

The ability of *Plasmodium falciparum* to evade immune response came from its capacity to change into many different allelic forms of its antigenic proteins while still maintaining the biological function. This diversity occurs through random mutations when a population undergoes frequent constrictions (a genetic bottleneck, by drug selection pressure) and subsequent clonal expansion (Sutton *et al.*, 2010). Furthermore, genetic recombination occurs in mosquitoes during the diploid short phase following fertilisation. When a mosquito ingests gametocytes, from genetically different parasites, meiotic recombination favours the exchange of genetic materials (cross- fertilisation) finally producing new allelic combinations and haplotypes (Pau and Day, 1998).

This genetic diversity can be reduced by immune (but not always) or drug pressure, which thus selects parasites that harbour genes conferring resistance to antimalarials drugs. The inherent variability of *P. falciparum* is particularly prevalent in merozoite surface antigens being targeted for malaria vaccines (Sakihama *et al.*, 2006). This provides multiple effective evasion and drug resistance mechanisms for the parasite.

I.5.1. Immune pressure and evasion of *P. falciparum*

This evasion is attibuted to the presence of non-synonymous polymorphisms found within the csp, msp and glurp genes concerned by these variations. *P. falciparum* may use repetitive, immuno-dominant epitopes as a mechanism to evade the immune response of the human host (Anders, 1986) and many of malaria antigens contain tandem arrays of relatively short sequences. Also, maintenance of degeneracy (one of the main properties of the genetic code) within a repeat set, and the existence of crossreacting epitopes in many genes of *P. falciparum* as a consequence of short repetitive sequences and the biased amino acid composition, have been suggested to interfere with the normal maturation of high affinity antibodies.

I.5.2. Immunogenicity

There is an induced immune response at every stage of the life cycle of malaria parasite, meaning the implication of different specialized proteins. It is obvious that these different responses call for different types of epitopes and single nucleotides polymorphisms (SNPs) that can help measuring the ability of an antigen to induce an immune response (immunogenicity) of these proteins. The immune response induced by sporozoites/stage-specific proteins will as well be higher compared to that of others (Krzyczmonik *et al.*, 2012). Immunogenicity can also be ascribed to compensatory mutations that when selected increase the virulence of the parasite than that of the normal wild type as described below in the presence of continuous drug pressure.

I.5.3. Drug pressure

Resistance is defined as the ability of a parasite to survive or multiply in the presence of concentrations of a drug that normally destroy parasites of the same species or prevent their multiplication. The WHO has defined three levels ; following treatment, parasitaemia clears but a recrudescence occurs ; following treatment, there is a reduction but not a clearance of parasitaemia and following treatment, there is no reduction in parasitaemia (WHO, 2006). Drug pressure is one of those factors that lead to mutation in the malaria parasite. This is mainly related to host parasite interactions and development of protective mechanisms by the parasites. More specifically, because of deletions, insertions or substitutions in certain genes, single nucleotide polymorphisms (SNPs) have been identified in *P. falciparum* clones which confer resistance to antimalarial drugs.

I.6. Resistance

The efficacies of many antimalarial drugs are limited by drug resistance, and recent evidence suggests that parasites are becoming resistant to the newest agents. However, the extent of resistance varies, such that in many cases drugs with resistance concerns are nonetheless offering good effectiveness for the treatment and control of malaria. Resistance has been described for nearly all available drugs, as is discussed below. For many drugs the extent of resistance is uncertain and mechanisms of resistance are unknown. Resistance can be assessed by clinical trials comparing antimalarial efficacies of different agents, in vivo/in vitro

assessment of sensitivities of cultured *P. falciparum*, evaluation of genetic polymorphisms associated with resistance, or by assessing the selective pressure of antimalarial treatment on subsequent infections. Studies considering all of these factors have shed light on the extent of resistance and on the mechanisms of resistance.

Resistance mediated by transporter mutations

The *P. falciparum* genome encodes multiple predicted transporters (Mu *et al.*, 2003) Polymorphisms in transport proteins can mediate resistance to many agents active against cancer and infectious diseases via enhancing efflux of the drugs from cells (Borges-Walmsley *et al.*, 2003). It appears that a number of plasmodial proteins transport different drugs and those polymorphisms in these proteins may impact on drug sensitivity (Picot *et al.*, 2009).

I.6.1. *Plasmodium falciparum multi drug resistance* 1 (P*fmdr*1)

Polymorphisms in the *P. falciparum* multidrug resistance-1 (pfmdr1) gene, which encodes the P-glycoprotein homolog, impact on sensitivity to multiple antimalarial drugs. In *P. falciparum*, the function of the pfmdr1 product is unknown (Foote *et al.*, 1990), but the protein localizes to the membrane of the food vacuole, the site of action of a number of drugs, suggesting that it is a drug transporter. Data on associations between pfmdr1 polymorphisms and drug sensitivity are complex (Sanchez *et al.*, 2010), but overall suggest that changes in pfmdr1 sequence or copy number alter transport of multiple drugs in or out of the parasite food vacuole, with individual polymorphisms leading to opposite effects on different drugs (Valderramos *et al.*, 2006). Mutations at pfmdr1 N86Y and D1246Y (for this and other P. falciparum genes, wild type sequence is based on the 3D7 reference strain), which are common in Africa, have been linked to decreased sensitivity to chloroquine and amodiaquine, but increased sensitivity to lumefantrine, mefloquine, and artemisinins (Sharom. 2011).

I.6.2. Plasmodium *falciparum chloroquine resistance transporter* (P*fcrt)*

Soon after the identification of pfmdr1, it became clear that polymorphisms in this gene are not the primary mediators of chloroquine resistance. Subsequently, analysis of progeny of a genetic cross between chloroquine sensitive and resistant strains led to the identification of *Pf*crt, which encodes a food vacuole membrane protein that is predicted to be a member of the drug/metabolite transporter superfamily. The function of *Pf*crt is unknown, but apparently essential, as disruption of the gene has not been possible, *Pf*crt is highly polymorphic

(Cowman *et al.,* 1991), but one single nucleotide polymorphism (SNP), K76T, is the primary mediator of chloroquine resistance. The 76T mutation appears to act principally by increasing the export of chloroquine from the food vacuole, but the mechanism of pfcrt 76T-mediated chloroquine resistance is incompletely understood. Other pfcrt SNPs always accompany 76T in field isolates, and these likely encode compensatory mutations that allow parasites containing 76T to maintain adequate fitness; some other SNPs may also contribute directly to the drug resistant phenotype (Koenderink *et al.,* 2010).The 76T mutation also mediates decreased sensitivity to monodesethylamodiaquine, and studies with genetically modified parasites have shown it to mediate increased susceptibility to mefloquine and artemisinins, suggesting the same reciprocal relationship between sensitivities to aminoquinolines and other drugs as described for certain pfmdr1 polymorphisms (Picot *et al.,* 2009).

I.6.3. *Plasmodium falciparum* multidrug resistance protein-1 (*Pfmrp*-1)

Plasmodium falciparum multidrug resistance protein-1 (*Pf*mrp1) is a member of the ABC transporter superfamily. In studies of culture-adapted P. falciparum, SNPs in pfmrp1 were linked to decreased sensitivity to chloroquine and quinine. Two SNPs that appear to be common in African parasites, I876V and K1466R, were selected by prior treatment with artemether/lumefantrine and sulfadoxine/pyrimethamine, respectively, although these SNPs were not associated with altered drug sensitivity in African isolates (Price *et al.,* 2004). *Pf*mrp1 mutations appear to differ between continents; some SNPs in northeast Myanmar isolates were associated with reduced susceptibilities to chloroquine, mefloquine, pyronaridine, and lumefantrine. Disruption of the pfmrp1 gene yielded parasites with diminished growth and increased sensitivity to chloroquine and other drugs, suggesting a role for this protein in the efflux of antimalarial drugs from the parasite and in parasite fitness (Sidhu *et al.,* 2002).

I.6.4. *Sodium transporters*

Quantitative trait locus analysis identified three genes predicted to play roles in the responsiveness of *Plasmodium falciparum* to quinine, *Pf*crt, *Pf*mdr1, and *Pf*nhe1, which encode a putative sodium–hydrogen exchanger and are highly polymorphic. *Pf*atp4 encodes a P. falciparum plasma membrane protein that appears to be a sodium efflux pump. Recent studies have shown that three different classes of potent antimalarial compounds, spiroindolones, pyrazoleamides, and dihydroisoquinolones, all target *Pf*atp4. Mutations in

pfatp4 have been linked to altered sensitivity to these candidate antimalarials (Picot *et al.,* 2009).

I.6.5. *Resistance to quinine*

Resistance to quinine, the oldest antimalarial drug, was reported first in Brazil and later in southeast Asia.Quinine resistance is associated with polymorphisms in several transporters. As stated earlier, SNPs in *Pf*mdr1, *Pf*crt, and *Pf*mrp1 are linked to decreased sensitivity to quinine. In addition, *Pf*mdr1 gene amplification can also lead to quinine resistance (Koenderink *et al.,* 2010).

I.6.6. *Resistance to antifolates*

The parasite-specific antimetabolite, pyrimethamine, is usually discussed in combination with its partner drug sulfadoxine (known as SP or Fansidar). Pyrimethamine was first used as an individual drug, but resistance was seen within a year in both *Plasmodium vivax* and *Plasmodium falciparum*. The combination of sulfa drugs and pyrimethamine proved to be potent in the laboratory, as well as in the field against chloroquine-resistant uncomplicated malaria but, again, resistance appeared rapidly in the Asia Pacific regions in the late 1970s, as well as in South America (Duraisingh *et al.,* 1998).

Molecular genetic studies attribute pyrimethamine and sulfa resistance to mutations in the genes coding for the target enzymes DHFR and DHPS. These markers have been useful tracking sulfadoxine/pyrimethamine resistance across the globe, and show particular promise with new multiplex strategies. In the 1990s, sulfadoxine/pyrimethamine found increasing use in Africa to treat widespread chloroquine-resistant malaria, before sulfadoxine/pyrimethamine resistance followed. Sulfadoxine/pyrimethamine is no longer recommended as a first-line drug for the treatment of *Plasmodium falciparum*. However, it continues to be used in ACTcombinations in most parts of India, for intermittent preventive therapy in pregnant women in Africa, and for seasonal malaria chemoprevention in children in the sub-Sahel of Africa, although widespread resistance limits these interventions (Reed *et al.,* 2000).

I.6.7. *Resistance to artemisinin family drugs*

Since artemisinins play an indispensable role in current malaria therapies, artemisinin resistance has received wide recent attention. In the Cambodia–Thailand border region of Southeast Asia, an epicenter of antimalarial drug resistance, declining efficacy of the

artesunate/mefloquine combination was noted, and clinical resistance to artesunate, manifested as delayed clearance of parasitemia after therapy, but not generally as full-blown treatment failure, was documented in 2008. In field-based studies, genome-wide association studies identified regions on chromosome 13 linked to delayed parasite clearance. Using a combined resistance selection and genomic approach, Ariey and others identified mutations in the propeller domain of the *Plasmodium falciparum* kelch (K13) gene associated with delayed parasite clearance after artemisinin therapy in southeast Asia (Nosten and White, 2007).

I.6.8. *Resistance to Malarone*

Atovaquone is a potent inhibitor of electron transport, and studies identified the target of this drug as the critical quinone-binding sites of cytochrome when the drug is used alone, resistance develops rapidly and recrudescence after therapy is common. Resistance is conferred by single-point mutations in the cytochrome b (*Pf*cytb) gene. *Pf*cytb mutations 268S and 268N were associated with Malarone treatment failure. However, treatment failure has also been reported in the absence of these mutations (Spillman *et al.*, 2013).

I.7. Mechanisms of resistance to antimalarial drugs

Several studies have showed that factors that increase resistance to antimalarials include: Non-compliance to protocol regimens, auto medication, irrational prescription of antimalarials, absence or non-respect of national policy, illicit sale of antimalarials, inadequate concentration of medication in the system, usage of monotherapy with a long half-life (Sulfadoxine-Pyrimethamine, Mefloquine), bad quality antimalarials, genetic heterogeneity of *P. falciparum*, rapid production of gametocytes carriers of gene resistance.

In general, resistance appears to occur through spontaneous mutations that confer reduced sensitivity to a given drug or class of drugs. For some drugs, only a single point mutation is required to confer resistance, while for other drugs, multiple mutations appear to be required. Provided the mutations are not deleterious to the survival or reproduction of the parasite, drug pressure will remove susceptible parasites while resistant parasites survive. Single malaria isolates have been found to be made up of heterogeneous populations of parasites that can have widely varying drug response characteristics, from highly resistant to completely sensitive (Thaithong *et al.,* 1983). Similarly, within a geographical area, malaria infections

demonstrate a range of drug susceptibility. Over time, resistance becomes established in the population and can be very stable, persisting long after specific drug pressure is removed. The biochemical mechanism of resistance has been well described for chloroquine, the antifolate combination drugs, and atovaquone.

I.7.1. Chloroquine resistance

As the malaria parasite digests haemoglobin, large amounts of a toxic by-product are formed. The parasite polymerizes this by-product in its food vacuole, producing non-toxic haemozoin (malaria pigment). It is believed that resistance of *P. falciparum* to chloroquine is related to an increased capacity for the parasite to expel chloroquine at a rate that does not allow chloroquine to reach levels required for inhibition of haem polymerization (Foley and Tilley, 1997). This chloroquine efflux occurs at a rate of 40 to 50 times faster among resistant parasites than sensitive ones (Krogstad *et al.,* 1987). Further evidence supporting this mechanism is provided by the fact that chloroquine resistance can be reversed by drugs which interfere with this efflux system (Martin *et al.,* 1987). It is unclear whether parasite resistance to other quinoline antimalarials (amodiaquine, mefloquine, halofantrine, and quinine) occurs via similar mechanisms (Foley and Tilley, 1997).

I.7.2. Antifolate combination drugs

The principal antifolate drugs used against malaria are Pyrimethamine, Proguanil (metabolized in vivo to the active form Cycloguanil) and the sulfa drugs, the most important of which are the sulfonamide, Sulfadoxine, and the sulfone, Dapsone. Antifolate combination drugs, such as sulfadoxine + pyrimethamine, act through sequential and synergistic blockade of 2 key enzymes involved with folate synthesis. Pyrimethamine and related compounds inhibit the step mediated by dihydrofolate reductase (DHFR) while sulfones and sulphonamides inhibit the step mediated by dihydropteroate synthethase (DHPS) (Bruce-Chwatt *et al.,* 1986). Specific gene mutations encoding for resistance to both DHPS and DHFR have been identified. Specific combinations of these mutations have been associated with varying degrees of resistance to antifolate combination drugs (Plowe *et al.,* 1995).

Following studies of bacterial systems from the 1940s, it was established that Pyrimethamine and Cycloguanil target the dihydrofolate reductase (*dhfr*) activity of the parasite's bifunctional dhfr-thymidylate synthetase (TS) protein, whereas the sulfa drugs affect the dihydropteroate synthetase (*dhps*) activity of the bifunctional hydroxymethylpterin pyrophosphokinase

(HPPK)-*dhps* protein; all of these drugs acting as competitive inhibitors of the natural substrates. There are specific point mutations in the *dhfr* gene, known to be associated with resistance to Pyrimethamine reducing the affinity of binding to *dhfr* (Muller & Hyde, 2010). The safety and efficacy of the drugs that target this enzyme therefore depend on several hundred-fold different in their binding to the parasite and human orthologs. Similarly, resistance to Sulfadoxine most commonly involves the changes A437G, K540E and A581G in the DHPS enzyme. The most frequent resistant combination in *dhps* currently prevalent in Africa is the double-mutant form A437G, K540E, which, when found together with the triple-mutant form of *dhfr*, yields the so-called quintuple mutant, a pattern resulting in parasites that are highly resistant to SPand a strong predictor of clinical failure. The significance of such changes with respect to quantitative resistance levels to the antimalarials will of course need to be established by the types of experiments used to causally link the original mutations to resistance (Muller & Hyde, 2010; Travassos & Laufer, 2009).

I.7.3. Atovaquone

Atovaquone was initially tested against murine infections with *Pneumocystis carinii*, *Toxoplasma gondii*, *Cryptosporidium parvum* and *Leishmania donovani* before it was introduced as a chemotherapeutic agent against malaria. It is now administered as chemoprophylaxis and stand-by treatment in combination with the antifolate Proguanil (PG) under the brand name Malarone™.

Atovaquone is a substituted hydroxynaphthoquinone that acts through inhibition of electron transport at the cytochrome bc1 complex in the mitochondrion, and impedes pyrimidine biosynthesis, an essential process which leads to parasite death (Ittarat et al., 1994). Although resistance to atovaquone develops very rapidly when used alone (linked to the appearance of mutations in the *cyt b* gene of the parasite), when combined with a second drug, such as proguanil which promotes a synergistic effect, and most probably not due to its role as an antifolate but rather by its exerting a direct impact on the mitochondrial membrane potential. Nevertheless, even such combination therapy has not entirely prevented further treatment failure and resistance mutations affecting position Y268 have been found in field isolates (Muller & Hyde, 2010).

I.7.4. Artemisinins

Multiple approaches and measurements have been used to assess artemisinin resistance in laboratory studies. Since the Ring-stage Survival Assay (RSA) provides a gold standard of in vitro artemisinin resistance that has been validated against *in vivo* resistance, it provides a robust index to simplify comparisons between findings across multiple studies. Since variations may arise due to differences in strain background, we used 3D7 strain data to compare the effect of K13C580Y mutation (responsible 80% of resistant parasites in SE Asia) as well as proposed determinants of K13-independent resistance. Transgenic elevation of parasite phosphatidylinositol-3-phosphate (PI3P) (via expression of VPS34) in absence of K13 mutation, induced greater than a log change of resistance in the RSA and comparable to levels induced by K13C580Y. Moreover, expression of VPS34 myc amplified a proteome that significantly overlapped with upregulated genes of the *in vivo* clinical transcriptome.

In vitro chemo-selected resistant parasites also independent of K13 showed much lower levels of resistance and some association with the *in vivo* clinical artemisinin resistance transcriptome. But transgenic expression of the high value candidate genes of the oxidative stress and protein damage, did not result in an RSA survival rate of 2%.

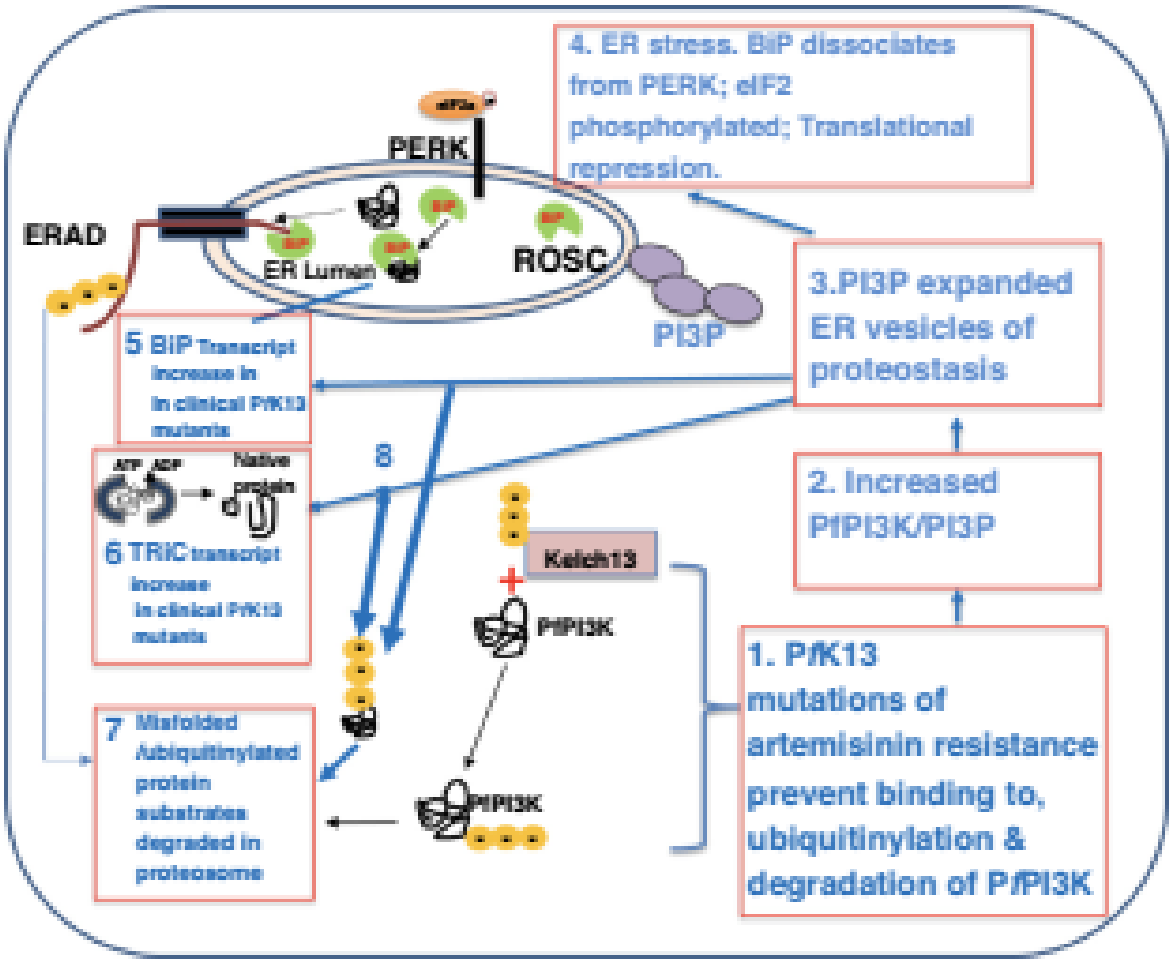


Figure 4: Mechanisms of resistance to antimalarial drugs **(Source:** Rocamora F, Zhu L, Liong KY, Dondorp A, Miotto O, et al. (2018) *Oxidative stress and protein damage responses mediate artemisinin resistance in malaria parasites. PLOS Pathogens 14(3)*: e1006930. https://doi.org/10.1371/journal.ppat.1006930)

In sum, amplification of *PI3P* vesiculation as modeled in Figure 4 (steps 1–3) remains the stronger predictor of artemisinin resistance. Its transcriptional imprint maybe upregulation of ER vesiculation, parasite oxidative stress and protein damage pathways via the unfolded protein response (UPR). Individual components of adaptive responses against oxidative stress and protein damage may act in concert within PI3P proteostatic vesicles or in addition to them, to confer resistance (Figure 4, steps 4–6). Therefore, although in vitro-selected artemisinin-resistant parasites were not analyzed for levels of PfPI3K-protein or PI3P-lipid vesicles (Rocamora *et al.,* 2018), their transcriptional profiles support the model in figure 4 that expansion of homeostatic ER vesiculation is a major determinant of artemisinin resistance. The model also explains additional findings from recent studies that PERK (also known as PK4; step 4) in the presence of artemisinins, phosphorylates eukaryotic initiation

factor-2a leading to translation arrest and the induction of latency (Zhang *et al.*, 2017), providing a high level of proof that Plasmodium manifests at least one (of 3) well defined effector arms of the UPR of eukaryotes in artemisinin resistance.

I.8. Metabolism of antimalarials drugs

When drugs enter the body, their destiny is determined by the absorption, distribution, metabolism and elimination steps. The majority of pharmacogenetic differences have been characterized at the molecular level until now for genetic variations in enzymes responsible for drug metabolism. The pharmacokinetics was then the first field of clinical research to apply pharmacogenomics and is currently the most active in this regard. Drug metabolism occurs in 2 phases; phase I reactions which are nonsynthetic involve formation of a new or modified functional group or cleavage (oxidation, reduction, hydrolysis) and phase II reactions which are synthetic, involve conjugation with an endogenous substance (eg, glucuronic acid, sulfate, glycine) (MSD, 2016).

Phase I metabolism is the functionalization phase reactions involving oxidation, reduction and hydrolysis of xenobiotics. Most enzymes involved in the metabolism and elimination of drugs are part of the cytochrome P450 (CYP450). Phase II metabolism is the conjugation phase for glucuronidation reactions, sulfonation and acetylation. N-acetyltransferase type 2 (NAT2), was one of the first phase II enzymes discovered as polymorphic. This (NAT-2) catalyzes the transfer of an acetyl group from acetyl coenzyme A to certain drugs and other xenobiotics with arylamine structure (Bouquier, 2003). The active enzyme detoxifies drugs such as isoniazid, anti arrhythmic drug proainamide (PA), anti-inflammatory drug 5-aminosalicylic acid (5-ASA), and drugs used for the treatment of malaria. Single nucleotide polymorphisms (SNP) on NAT2 gene alter drug pharmacokinetics; this was demonstrated in a Cameroonian population (Bouquier, 2003). In the other hand, the influence of non-genetic factors, such as food intake, age, sex or body weight, on the pharmacokinetics of antimalarial drugs was also demonstrated. Antimalarial treatment responses allow distinguishing three phenotypes as: slow, intermediate and fast acetylators (Bouquier, 2003).

I.8.1. Selected data on clinical trial studies based on antimalarial drugs

In 2008, a study by Wan and collaborators, on the Efficacy of dihydroartemisinin-piperaquine (DP) and artemether-lumefantrine (AL) in the treatment of uncomplicated falciparum malaria in Hainan Province (China), demonstrate that both combinations are at high cure in an area with high level resistance of *Plasmodium falciparum* to chloroquine (Wang *et al.*, 2008),

In 2009, a study by Nguyen and collaborators on the Pharmacokinetics and *ex vivo* pharmacodynamic antimalarial activity of dihydroartemisinin-piperaquine in patients with uncomplicated falciparum malaria in Vietnam demonstrate that Dihydroartemisinin is responsible for most of the *ex vivo* antimalarial activity of dihydroartemisinin-piperaquine (Nguyen *et al.*, 2009).

In 2014, a study by Zani and collaborators on the evaluation of the effectiveness and safety of DHA-P compared to other ACTs for the treatment ofuncomplicated *P. falciparum* malaria in adults and children, in line with the WHO 'Protocol for assessing and monitoring antimalarial drug efficacy demonstrate that over 28 days of follow-up, DHA-P versus artemether-lumefantrine in Africa is superior to artemether-lumefantrine at preventing further parasitaemia and that in the trial from South America, there were fewer recurrent parastaemias over 63 days with artesunate plus mefloquine. Finaly, this DHA-P is associated with less nausea, vomiting, dizziness, sleeplessness (Zani *et al.*, 2014).

In 2015, Rapid diagnosis and effective treatment were considered as the cornerstones of malaria control and artemisinin-based combination therapy (ACT) is currently the main anti-malarial drugs used for case management. After ACT deployment due to widespread parasite resistance to the cheap and widely used anti-malarial drugs, chloroquine and sulphadoxine/ pyrimethamine, the World Health Organization recommends regular surveillance to monitor the efficacy of the new drugs (Shayo, 2015).

I.8.2. Role of Cytochromes and *NAT2* in the metabolism of antimalarial drugs

With the current situation of malaria in Africa, the large quantity of existing molecules which circulate, its irrational uses after dangerous presciptions help to cure the disease, but at the same time, can persist in the body and accumulate at a very toxic level, generate unwanted drug reactions, or cause other illnesses. Like other drugs, antimalarials need to be metabolized, detoxified and eliminate from the body as quickly as possible.

Role of Cytochromes

The major causes of interindividual and intraindividual variability in CYP activity are environmental factors (inducers and inhibitors), biological factors (gender, disease, and circadian rhythms), and genetic polymorphisms in CYP450 genes and their regulators. There are large variations between individual CYP450 isoforms in terms of their susceptibility to these mechanisms (Zanger *et al.*, 2008). Overall, 57 CYP450 genes and 58 pseudogenes have been identified, 42 of which play a role in the metabolism of both exogenous xenobiotics and endogenous substances (e.g., steroids and prostaglandins), and 15 of which are involved in the metabolism of drugs in humans (Zanger *et al.,* 2008). CYP450 genes are highly polymorphic and can exhibit clinically significant genetic polymorphisms. In general, CYP3A4/5, CYP2D6, CYP2C9, CYP2C19, CYP2A6, CYP2B6, and CYP2C8 are the most important and most studied metabolic enzymes (Table 2).

CYP2C8 accounts for approximately 7% of total hepatic content and plays a vital role in the metabolism of pioglitazone, amiodarone, paclitaxel, chloroquine, verapamil, and ibuprofen. It also plays a secondary role in the metabolism of fluvastatin, amitriptyline, diclofenac, omeprazole, and carbamazepine. The metabolism of paclitaxel to 6α-hydroxypaclitaxel, which is essentially inactive, has been used as an index of CYP2C8 activity in vitro (Harris *et al.*, 1994). The most common CYP2C8 variants are CYP2C8*2 (rs11572103, 11054A >T), CYP2C8*3 (rs11572080, 2130G >A and rs10509681, 30411A >G), and CYP2C8*4 (I264M substitution); they lead to decreased enzyme activity. CYP2C8 is primarily responsible for the hydroxylation of R-ibuprofen; CYP2C9, for S-ibuprofen (Zanger *et al.*, 2008).

Role of N-acetyltransferase 2

N-acetyltransferase 2 is a gene that encodes an enzyme that functions to both activate and deactivate arylamine and hydrazine drugs and carcinogens. It's a phase II enzyme that participates in the detoxification of a plethora of hydrazine and arylamine drugs. Catalyzes the N- or O-acetylation of various arylamine and heterocyclic amine substrates and is able to bio activate several known carcinogens. It catalyzes the transfer of the acetyl group from the cofactor acetyl coenzyme A (Acetyl-CoA) to the nitrogen terminal of the drug (Toure *et al.*, 2012) (figure 5).

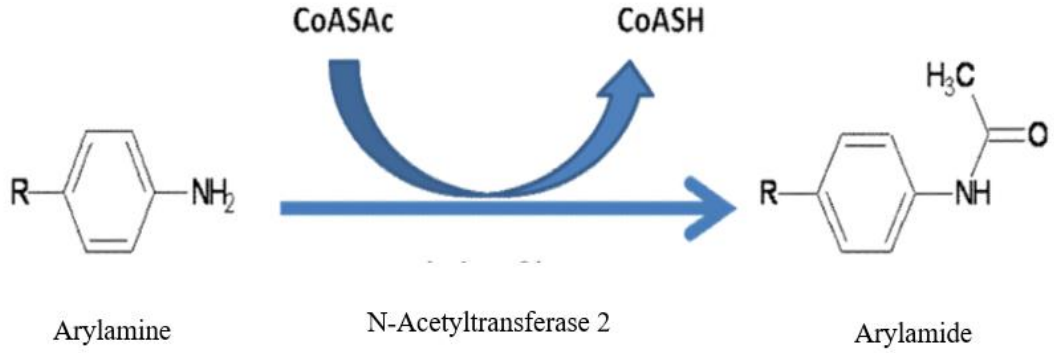


Figure 5: N-Acetylation of xenobiotics by NAT2 (Toure *et al.*, 2012).

Polymorphisms in this gene are responsible for the N-acetylation polymorphism in which human populations segregate into rapid, intermediate, and slow acetylator phenotypes. Polymorphisms in this gene are also associated with higher incidences of cancer and drug toxicity (Kurashima, 2016). A second arylamine N-acetyltransferase gene (NAT1) is located near this gene (NAT2). Diseases associated with NAT2 include Nat2-Related Altered Drug Metabolism and Isoniazid Toxicity. Among its related pathways are Metabolism and Caffeine Pathway, Pharmacokinetics. Annotations related to this gene include *acetyltransferase activity* and *arylamine N-acetyltransferase activity*. An important paralog of this gene is NAT1. In humans NAT2 is located in the NAT cluster that comprises 230 kb and includes two functional genes, NAT1 and NAT2. In other species the number of NAT genes range from 0, for instance in dogs, to 4 for instance in chicken (Khan *et al.*, 2013).

Figure 6: Structure of the human NAT2 gene (Khan *et al.*, 2013)

The human NAT2 gene has two exons but the coding region, spanning 870 bp is located in exon 2. Functionally active NAT2 enzyme can be obtained after transient heterologous transfection of the open reading frame only, indicating that exon 1 is not necessary to obtain functional enzyme. NAT enzymes have been identified in several vertebrate and

microorganism species. NAT2 proteins differ among species. However, common features include an 83 amino acid N-terminal domain containing five alpha-helices and a short beta-strand; a second domain consisting of nine beta-strands and two short helices; and a third alpha/beta lid domain with four beta-strands and an alpha-helix (Jhon *et al.,* 2009).

Expression, localization and function

NAT2 has a restricted expression profile with the highest levels of protein and mRNA being detected in the liver, small intestine and colon. The transcription start site for human NAT2 has been recently localized between 30 and 101 bp upstream of the non-coding exon, with the most frequent TSS located at position -64 relative to exon 1. The region containing the NAT2 transcription start site shares an 85% sequence homology to the region of human NAT1 containing the major transcription start site for NAT1. The functional elements of the NAT2 promoter sequence have not been characterized to date (Jhon *et al.,* 2009). In addition, the promoter sequence appears to be highly polymorphic. Arylamine N-acetyltransferase 2 is a cytosolic enzyme. NAT2 is a phase II enzyme that participates in the metabolism of numerous primary arylamine and hydrazine drugs and carcinogens. In addition to their N-acetylation catalytic activity (Jhon *et* al., 2009).

Mutations

Seven major single nucleotide polymorphisms that occur isolated or combined have been described in the NAT2 gene. These affect the positions 191, 282, 341, 481, 590, 803 and 857. In addition, rare SNPs affecting the positions 111, 190, 364, 411, 434, 499, 795, 845 and 859 have been described although their frequencies are unknown (Jhon *et* al., 2009). Critical gene variants leading to slow acetylation capacity contains mutations at positions 191, 341, 590 or 857. Since some genotypes can be due to the presence of different combinations of haplotypes leading to ambiguous phenotype prediction, haplotype reconstruction is often necessary to clarify ambiguous genotype data (Teixeira *et al.,* 2013).

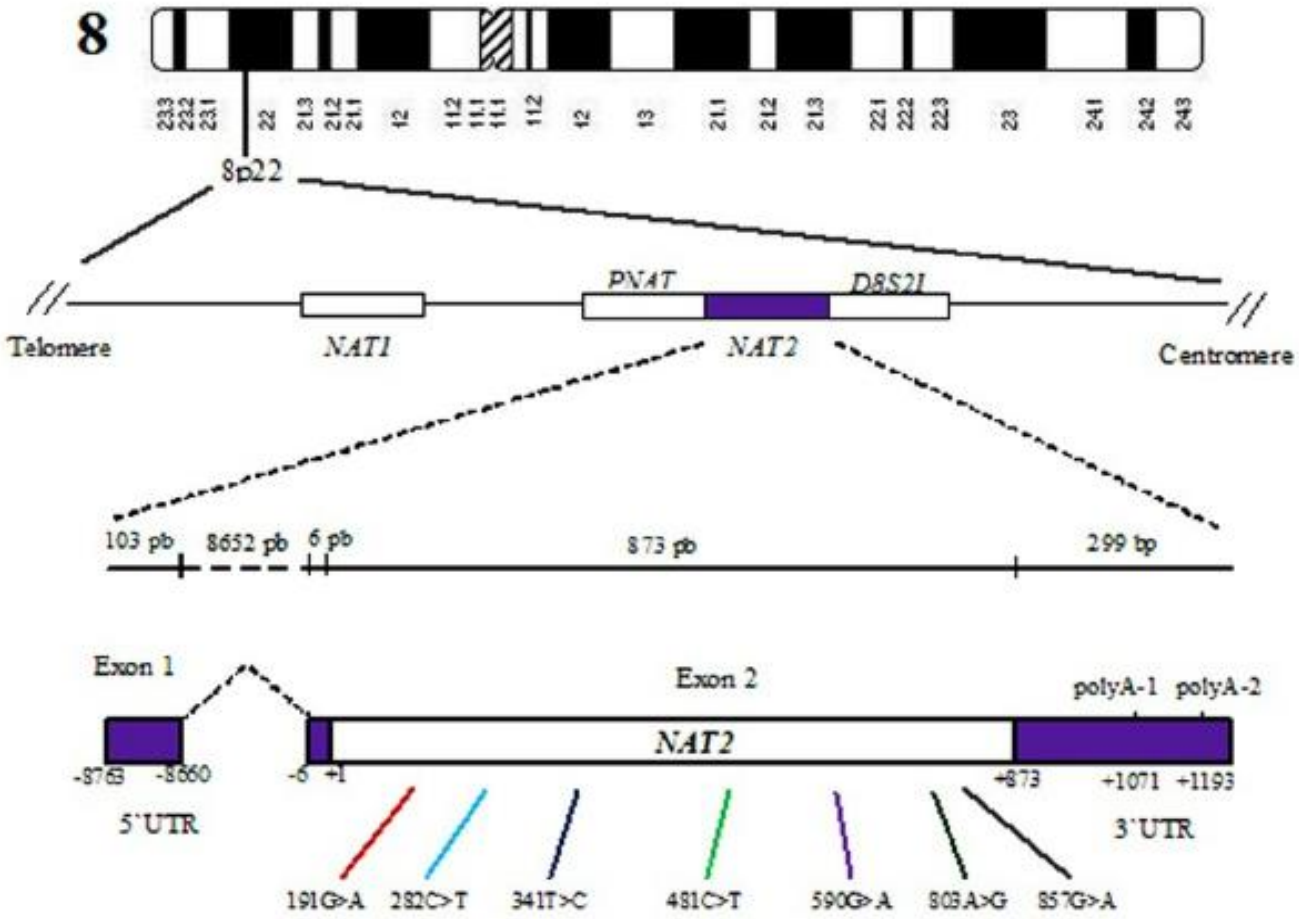


Figure 7: Schematic representation of NAT genes (Teixeira *et al.*, 2013)

D8S21 represents a polymorphic marker situated in the NAT2 locus

Table 1: Antimalarial metabolization (Naazneen *et al.*, 2015)

N°	Antimalarials	Description	Mode of Action	Drug metabolyzing Enzyme
01	**Primaquine**	effective against the exo-erythrocytic stages of *Plasmodium vivax* and *Plasmodium ovale* and gametocytic stages of *Plasmodium falciparum* (Edwards et al., 1993).	disrupts the metabolic processes of *Plasmodium* mitochondria by interfering with the function of ubiquinone as an electron carrier in the respiratory chain *(Hill et al., 2006).* The main metabolites of piperaquine are carboxylic acid	metabolic pathways of piperaquine have not been characterised, (Lee et al., 2004, Tarning et al., 2006) identifi cation of N-oxydated, hydroxylated, and O-demethylated metabolites suggests an involvement of cytochrome P450 enzymes. primarily converted to metabolite carboxyprimaquine by CYP1A2. (Hill et al., 2006*)*, and is also metabolized by CYP3A4 *(Kerb et al., 2009).*
02	**Artemisininderivatives**	extracted from *Qinghao* (blue-green herb) (Hsu, 2006) and is used in severe malaria and is effective against all the blood stages of *Plasmodium falciparum* including the youngest (ring form) stage of the parasite *(WHO, 2006).* A combination of lumefantrine and artemether is a useful treatment for uncomplicated falciparum malaria, and is used as a standby emergency therapy for travellers*(Novartis Pharma, 2006)*	The endoperoxide bridge of Artemisinin interacts with haem to produce free radicals that alkylate protein and damage the micro-organelles and membrane of the parasite. It also interrupts with the haemoglobin catabolism system causing inhibition of haemoglobin degradation and polymerization of haem to haemozoin which is used by parasite as its nutritional requirements *(Pandey et al., 1999).* Lumefantrine is activated, mainly by CYP3A4, to form desbutyllumefantrine, which has fi ve-times to eight-times higher antiparasitic activity in vitro although less than 1% of the exposure of the parent compound. (Novartis Pharma, 2006)125	mainly metabolized to dihydroartemisinin by CYP2B6(Malhotra et al., 2006). However, the other metabolizing enzymes also involved in the metabolism of artemisinin are CYP3A4 and CYP2A6 (Svesson and Ashton, 1999). Thus, patients might beneft from faster activation by artemether-mediated CYP3A4 induction.
03	**Proguanil**	an antimalarial drug used for the treatment of malaria caused by *Plasmodium falciparum (Pang et al., 1989).*	- Proguanil inhibits dihydrofolate reductase (DHFR) enzyme essential for synthesis of parasite DNA (Boggild et al.,.	converted to its active metabolite Cycloguanil by CYP2C19. Additionally, CYP3A4 have a limited role in the metabolism of Proguanil to Cycloguanil (Brentano *et al.*, 1997, 2007)
04	**Dapsone**	is a 4, 4‟‟-diamnodiphenyl sulphone and widely used for the treatment of *Leprosy, Chloroquine resistant malaria and*	inhibits folic acid synthesis of parasite by inhibiting DHFR enzyme (Tingle *et al.*, 1997).	Dapsone is metabolized by CYP2C9 (Tingle et al., 1997), CYP3A4 (Adedayo *et al.*, 1998) and NAT2 (Louet *et al.*, 1999)

		Dermatitis Herpetitiformis. It is effective against the treatment of schizonticidal and gametocidal activity of *Plasmodium falciparum*, but does not act against asexual form of the *Plasmodium vivax*. (Saha *et al.*, 2003).		
05	**Amodiaquine** :	Amodiaquine is derived from quinoline and is more effective as compared to chloroquine against Plasmodium falciparum (Li et al., 2002).	The mode of action against the parasite is same as that of chloroquine, by inhibiting polymerization of haem and thus, inhibiting formation of toxic haemozoin. (Hombhanje et al., 2004).	Amodiaquine is converted to N-Desethylamodiaquin by CYP2C8 (Li et al., 2002).
06	**Chloroquine**	The quinoline containing antimalarials are effective against malaria parasite *Plasmodium falciparum* (Krogstad *et al.*, 1998).	Chloroquine is a dibasic drug, which diffuses down the pH gradient and accumulates in the acidic vacuole of the parasite *Plasmodium*. The high intravacuolar concentration of chloroquine inhibits the polymerization of the haem. As a result the haem which is released during haemoglobin breakdown builds up to poisonous levels, killing the parasite (Foley and Tilley, 1997).	Chloroquine is metabolized by CYP3A4 to N-desethylchloroquine (Cooper and Magware, 2008).
07	**Clindamycin**	Semi synthetic derivative of Lincomycin and is effective against many species including *Plasmodium* sp. (Lell and Kremsner, 2002).	Clindamycin slowly accumulates inside the apicoplast of *Plasmodium* sp. and killing it ultimately (Lell and Kremsner, 2002).	Clindamycin is metabolized by CYP3A4 to Clindamycin sulfoxide (Wynalda *et al.*, 2003,). CYP3A5 catalyse the formation of clindamycin sulfoxide and the minor metabolite N-desmethylclindamycin, 129 which both show in-vitro activity.(Seaberg et al.,1984)
	Tétracycline Doxycycline	Tetracycline, doxycycline, or clindamycin, in combination with quinine, are particularly eff ective against falciparum malaria. Clindamycin is also safe in children and pregnant women.(Kremsner et al.,2004)	Although the metabolism of tetracyclines is largely unknown, they are concentrated in the bile and excreted in the faeces and urine at high concentrations in a biologically active form.	Tetracycline is known to be transported in vitro by hepatic human organic anion transporter (hOAT) 2, and renal hOAT3 and hOAT4.128 CYP3A4 and, to a lesser extent,

	Atovaquone	Atovaquone in fi xed combination with proguanil had proved to be highly eff ective in preventing and treating falciparum malaria until the recent emergence of resistance. Several quinoline derivatives are in phase 3 studies or under registration as long-acting components for ACT.	Biliary excretion has also been suggested as the major route of elimination for atovaquone, which is not metabolised.127	
	Pyronaridine	These include piperaquine in combination with dihydroartemisinin or as triple therapy with trimethoprim, and pyronaridine combined with artesunate. (Ollario et al.,2009)	The main metabolites of pyronaridine are quinoneimine.	metabolic pathways of pyronaridine have not been characterised, 131,132 identifi cation of N-oxydated, hydroxylated, and O-demethylated metabolites suggests an involvement of cytochrome P450 enzymes. pyronaridine might exhibit variable absorption through P-glycoproteinmediated effl ux.(Crowe et al., 2006)
08	**Halofantrine**	A phenanthrene methanol derivative used as antimalarial drugs against uncomplicated chloroquine and multidrug resistant *Plasmodium falciparum* (Halliday *et al.*, 1995).	Halofantrine is blood schizonticides and affects only trophozoites and schizonts in the red blood cells. It binds to ferriprotoporphyrin IX in red blood cells affected by *Plasmodium* and form toxic complexes that damage the parasite membrane *(*Nothdruft, 1993).	Halofantrine is metabolized by CYP3A4 to N-debutylhalofantrine (Baune, 1999;).
09	**Mefloquine**	antimalarial is effective against the chloroquine resistant strains of *Plasmodium falciparum* (Riviere *et al.*, 1985).	The quinoline binds to high density lipoproteins (HDL) in the serum and delivered to the erythrocytes, where they interact with an erythrocyte membrane protein stomatin and are then transferred to the intracellular malaria parasite.	Mefloquine is metabolized by CYP3A4 (Khaliq *et al.*, 2001).
10	**Sulphadoxine**	is a sulphonamide and is effective against *Plasmodium falciparum*. It is used in combination with other drugs to treat	inhibits folic acid biosynthesis by blocking the formation of dihydrofolic acid and inhibiting dihydropteroate synthase (DHPS) enzyme (Ridley	Sulphadoxine is metabolized by NAT2 (Fuchs, 2004; Melmon, 2000).

		malaria ((Seaton *et al*., 2000). The antifolate sulfadoxine-pyrimethamine combination can be used with artesunate or Amodiaquine .	*et al.*, 2002). Sulfadoxine is excreted primarily unchanged in urine, whereas pyrimethamine is metabolised in the liver by unknown enzymes before renal excretion.	
11	**Quinine**	Quinine is metabolised by CYP3A4/-A5 to its primary metabolite, 3-hydroxyquinine, (Zhang et al., 1997, Mirghani et al., 2006), which has been shown ex vivo to contribute 5–10% of the antimalarial activity.(Pukrittayakamee et al., 1997) Formation of the minor metabolites (10*S*)-11-dihydroxydihydroquinine and 2Ľ-quininone is also dependent onCYP3A*4*, while the formation of (10*R*)-11-dihydroxydihydroquinine might be linked to CYP2C9.(Mirghani et al., 2002)	In-vitro studies with Caco2 cells suggest that quinine at therapeutic levels is an inhibitor and substrate of MDR1. (Hayeshi et al.,2006, Crowe et al.,2006) Increased brain concentrations of quinine have been seen in MDR1a-knockout mice and in wildtype mice after inhibition of P-glycoprotein with cyclosporine, verapamil, and mefloquine. (Pussard et al.,2007) Quinine might be a substrate for human OCTs. (Koepse et al., 2007) Since OCT1 is almost exclusively expressed in the liver,40 OCT1- mediated uptake might promote hepatic quinine metabolism, whereas pancreatic OCT2 might contribute to the quinine-related increase of insulin secretion	Substantial variation of quinine properties between individuals has been seen in children from Ghana with severe malaria (Krishna et al, 2001) One study has shown that 3-hydroxylation of quinine was substantially lower in healthy people from Tanzania harbouring the *CYP3A5*3/*3* low-expression genotype,. (Mirghani et al.,2006) an initial finding that awaits independent validation and evaluation of clinical relevance.

Table 2: Summary about the detailed information on human drug metabolizing genes. (Naazneen et al., 2015)

<table>
<tr><th colspan="4">CYP 450 1A FAMILY</th></tr>
<tr><td colspan="4">CYP1A1 and CYP1A2 that play a major role in the biotransformation of food and environmental pollutants.

The human CYP1A2 is involved in the metabolism of antimalarial primaquine and spans a gene length of 7758 bp (Chromosome 15q24.1, position75041184 – 75048941) with 7 exons that codes for protein of 516 amino acid (www.ncbi.nlm.nih.gov) and mainly expressed in the liver (Daly, 2003).</td></tr>
<tr><th colspan="4">CYP450 2B FAMILY</th></tr>
<tr><td colspan="4">only one member CYP2B6 play a significant role in the metabolism of artemisinin. This gene spans a gene length of 27058 bp (Chromosome 19q13.2, position 41497204-41524301) with 9 exon (www.ncbi.nlm.nih.gov) that codes for protein of 491 amino acid (www.ncbi.nlm.nih.gov)which is highly expressed in liver, but also expressed in the brain, small intestine, kidney and lung (Nagata, 2002).</td></tr>
<tr><th colspan="4">CYP 450 2C FAMILY</th></tr>
<tr><td>The human CYP2C has four members viz.,CYP2C18 , CYP2C19, CYP2C9, and CYP2C8 clustered at human chromosome 10q24.
These four subfamily shares less than 82% amino acid identity and significantly contribute in different ways to metabolize drugs.
CYP2C19, CYP2C9 and CYP2C8 were found to be most divergent.
The maximum homology is shared between CYP2C19 and CYP2C9 (90% homology), whereas, CYP2C8 share only 75%</td><td>CYP2C8 is more divergent among all CYP2C family members as revealed from protein sequences.
This gene involves in the metabolism of Amodiaquine and is mainly expressed in human liver with expression level of less than 1 (Nagata and Yamazoe, 2002).
The human CYP2C8 gene spans a gene length of 30 000 bp with 9 exons that code for 490 amino acid protein.</td><td>CYP2C9 is also one of the major CYP2C that comprised of one third of total hepatic P450 content. This gene plays a pivotal role in dapsone and spans a gene length of 50734 bp (Chromosome 10q24, Position 96698415-96749148) and contains 9 exons that codes for protein of 490 amino acid and mainly expressed in liver (www.ncbi.nlm.nih.gov).</td><td>CYP2C19 is the largest gene among human CYP450 involved in drug metabolism. The CYP2C19 gene plays a significant role in the metabolism of proguanil and spans a gene length of 90209 bp (Chromosome 10q24.1-q24.3, position 96522463-96612671) and contains 9 exons that codes for protein of 490 amino acid (www.ncbi.nlm.nih.gov), which is mainly expressed in the liver (Nagata and Yamazoe, 2002).</td></tr>
</table>

homology identity *(Kudzi et al., 2009).*	(www.ncbi.nlm.nih.gov,		
CYP 450 3 FAMILY			
(CYP3A):- The human CYP3A family composed of 25% - 30% of total hepatic cytochromes P450 *(Shimada, 1994).* The CYP3A gene family is the most important drug metabolizing gene which is involved in the metabolization of almost 60% of drugs. The CYP3A family comprised of 4 functional genes: **CYP3A4, CYP3A5, CYP3A7 and CYP3A43** located within 218 Kb region of chromosome 7q22.1 (www.ncbi.nlm.nih).	In addition to these four functional genes, two **psuedogenes CYP3A5P1 and CYP3A5P2** are also present *(Agarwal et al., 2008).* **CYP3A4** exhibit approximately 85% amino acid sequence similarity with **CYP3A5** and **CYP3A7** and approximately 75% amino acid sequence similarity with **CYP3A43** *(Domanski et al., 2000).*		**CYP3A4** gene is involved in metabolism of wide variety of antimalarial. It is located at chromosome 7q21.3-q22.1 and spans a gene length of 27592 bp with 13 exons (www.ncbi.nlm.nih,) that codes for protein of 502 amino acids which is mainly expressed in the liver and intestine. In adults CYP3A4 is the dominant CYP3A enzyme that plays a dominant role in the metabolism of numerous drugs *(Hirota et al., 2004).*
N ACETYL TRANSFERASE NAT			
The human **N-acetyltransferase** is a broad spectrum drug metabolizing enzyme that catalyzes N-acetylation and O-acetylation of equally respond to a particular drug which supports the genetic basis of drug metabolism			

I.9. Pharmacogenomics

Among others, age, enzyme induction or inhibition, and diseases (especially of the liver) are sources of variation in enzyme activity. Variation in the DNA sequence of genes encoding enzymes can abolish, reduce, or increase the expression and activity of an enzyme, and this can manifest as the "metabolizer" phenotype in an individual. Individuals who are homozygous for the two alleles coding for "normal" enzyme function are termed extensive metabolizers (homozygous EM or "wild-type"); those who are homozygous with two variant alleles resulting in inactive or absent enzymes are "poor metabolizers" (PM); those who are heterozygous manifest an intermediate metabolizer (IM) phenotype with reduced function (heterozygous EM). Intermediate and extensive metabolizers are often collectively referred as extensive metabolizers, especially in studies in which metabolizer status is assigned using phenotype.

Gene duplication or multiplication, as, for example, seen in CYP2D6, can result in "ultrarapid metabolizer" (UM) phenotype. Standard drug doses achieve normal concentrations and effect in homozygous EMs (which usually make up the largest proportion of the population), but they may be toxic in PMs (possibly in heterozygous EMs or IMs) and ineffective in UMs, who may require a higher dose to achieve therapeutic effective drug concentrations (Li *et al.*, 2011).

In general, the most important factor affecting drug action is the AUC at the site of action, reflecting the "metabolizer" phenotype; AUC is the best pharmacokinetic end point for the assessment of pharmacogenetic effects. However, even if there is a clear association between concentration and drug effect/toxicity, there are multiple factors that determine the clinical relevance of a functional polymorphism in a drug metabolizing gene (Gardiner *et al.*, 2006).

I.10. Pharmacokinetics

Pharmacokinetics is the branch of pharmacology that deals with the absorption, distribution, and elimination of drugs, while pharmacodynamics deals with the actions of drugs on the organism. Pharmacokinetics describes how the concentration of a dosed drug and its metabolites in body fluids and tissues changes with time. PK models the concentration-time profile using key parameters, such as volume of distribution (Vd), area under the curve

(AUC), clearance (CL), half-life (t1/2), maximum concentration (Cmax), and bioavailability (F). These parameters provide insights on how processes of the living system affect the drug concentration, including absorption, distribution, metabolism, and excretion. Owing to the dependence of PK parameters on the drug's properties, one application of PK in drug discovery is to derive insights about how the structure might be modified to improve the Pharmacokinetics parameters. Since efficacy (pharmacodynamics) and toxicity are related to Pharmacokinetics, pharmacokinetic/ pharmacodynamic (Pharmacokinetics / pharmacodynamics) models can be derived. These models are useful for planning and interpreting in vivo studies of efficacy and toxicity and planning human clinical trials (Williams *et al.,* 2008).

I.10.1. Variability of pharmacokinetics between individuals

Exposure to many drugs like atovaquone, piperaquine and lumefantrine is highly variable from one individual to another (Ezzet *et al.,* 2000). An important factor determining this variability is dietary fat (Roland *et al.,* 1997). This is common with highly lipophilic drugs whose bioavailability is increased by increased effective solubility or by postprandial increases in lipoproteins zhich cause an increase in the fraction of lipoprotein-bound drug in plasma (Wasan *et al.,* 2008).

It is very interesting to understand that the success of any drug treatment depends on the individual pharmacogenomic vulnerability as well as the sensitivity of pathogen to drug. By improving our deeper understanding and awareness of genetic variability in drug metabolizinggene, parasite biology and vector behavior, it is possible to reduce the emergence and spread of drug resistance as well as being helpful for the development of safe, cheap and efficient drug. The most common *CYP2B6* gene polymorphism, *CYP2B6*6,* is associated with raised plasma concentrations of efavirenz and nevirapine, as well as efavirenz-related neurotoxicity in individuals infected with HIV (Zanger *et al.,* 2008). Studies that have incubated Amodiaquine with human liver microsomes and recombinant cells expressing the most common *CYP2C8* gene polymorphisms, *CYP2C8*2* and *CYP2C8*3,* have shown a 50% decrease in the metabolic activity of *CYP2C8*2,* and an 85% decrease in the activity of *CYP2C8*3,* compared with *CYP2C8* wildtype (**1*) (Zanger *et al.,* 2008).

I.11. Quality of antimalaria medicines

I.11.1. Status antimalarials quality

Antimalarial drugs are widely distributed and self-prescribed (incorrectly and correctly) for many febrile episodes attributed to malaria mostly in endemic regions. Insufficient facilities for the control of antimalarial drugs quality, poor consumers' and health-workers' knowledge on these drugs, their cost, and the paucity of appropriate regulatory and punitive action makes these drugs attractive targets for counterfeiters (Barnes *et al.*, 2007; White *et al.*, 2008). Of the various public health consequences of poor antimalarial drugs quality, drug resistance is a particular concern. Low concentrations of active pharmaceutical ingredient in poor quality antimalarial drugs can result in subtherapeutic concentrations of the drug in vivo, which contributes to the selection of resistant parasites (Bains, 2013).

There are still no universally accepted definitions for different types of poor-quality drugs and national terminologies are diverse. Some nations, and some of those involved in intellectual property law, are concerned by the fact that the word counterfeit may mislead some legitimate generic drugs as such, which will lead to many debates about terminology. WHO's executive board documents use the term spurious/falsely-labelled/falsified/counterfeit medical products (Mishra *et al.*, 2016). Poor quality drugs are classify into three main types: **falsified** (fraudulently manufactured with fake packaging and usually no or a wrong active pharmaceutical ingredient); **substandard** (products resulting from poor manufacturing with no intent to deceive, usually with inadequate or too much active pharmaceutical ingredient); and **degraded** (good-quality drugs that are degraded by poor storage after leaving the factory) (White *et al.*, 2008; Florens *et al.*, 2002; Greenwood *et al.*, 2008).

1.11.2. Quality of ACTs

Artemisinin derivatives are the most effective drugs against malaria, and artemisinin-based combination treatments are the recommended first-line treatments for *P. falciparum* malaria (Bains *et al.*, 2013). Resistance or tolerance to artemisinin derivatives is characterized by slow rates of parasite clearance after treatment. Although a causal relation between poor quality artemisinin derivatives and artemisinin resistance has not been confirmed, modelling analyses suggest that under dosing of patients can play an important role in the spread of resistance.

Poor-quality antimalarial drugs are very likely to jeopardise the unprecedented progress and investments in the control and elimination of malaria made in the past decade.

According to malaria Medicines Venture (MMV) counterfeiting of antimalarial drugs is a multi-billion-dollar business, with very sophisticated operators and an excellent ability to evade detection by drug-regulatory authorities in Africa and other continents. This has developed the global trade in fake medicines: it potentially kills up to 100 000 people every year. (Nayyar *et al.,* 2012). Counterfeit traders mainly operate in developing countries. We agree with Nayyar and colleagues that production and distribution of counterfeit anti malarial drugs, or even any drug, should be deemed a crime against humanity. Therefore, countries must take extra measures to quash the manufacture of illegal and substandard malaria products, which endanger patients and put the effectiveness of authentic artemisinin at risk. Moreover, countries must also develop national capability to control the quality of drugs at the point of import and by random sampling in central, regional and peripheral levels of the procurement chain: this will enable drug-regulatory authorities to protect the national drug supply. In other to succeed, the development of innovative technologies shoul be established such as Sproxil, which uses short message service (SMS) technology helping consumers to protect themselves from counterfeit products (Nayyar *et al.*, 2012).

I.11.3. Quality control of medicines

The chief pharmacist responsible for a pharmaceutical structure must release batches of medicines in accordance with the requirements of the marketing authorization file for patients, in order to avoid risk related to safety, quality and efficiency. This responsibility needs the fully implementation of personnels involved in the different departments at all levels, but also suppliers of raw materials, including containers. The medicines distribution channels must also be compliant to quality. Thus, the attainment of these objectives required an appropriate quality assurance system adequately implemented and efficiently controlled. This system included the concept of Good Manufacturing Practices and Quality Control that implies active participation of managers and personnel from diverse services.

I.11.3.1. Quality Assurance

According to ISO 402-198, quality is define as: “the sum of properties and characteristics of a product or service which confer to it the aptitude to satisfy expressed or implicit needs”. According to European GMP, « Drug quality», refers to the quality to reach in order to fit

patients needs and the quality described in the file for market authorization (Alain *et al.*, 2001). Quality assurance according to ISO 8402-94 as “a set of pre established and systematic activities put in place within the quality system framework, expressed as needs to give appropriate confidence in what an entity will satisfy quality requirements”; for WHO Guidelines, quality assurance is a wide concept englobing all questions which influence the quality of a product individually or it overall environment.

I.11.3.2. Quality control of medicines

Quality Control of medicines is realized by implementing in a laboratory two types of testing: testing required by pharmacopeias: protocols and standards are designed in pharmacopeias and in the Module 3 of market Authorization dossier or the Common Technical Document (CTD); and testing not required by pharmacopeia*:* protocols and standards are designed only in the Module 3 of market Authorization dossier or the Common Technical Document (CTD). Testings required by pharmacopeias to control quality of solid dosage forms can be divided into 4 categories: Physical aspect of medicine; Pharmacotechnical tests; Physico chemical tests and Microbiological tests.

CHAPTER TWO :

METHODOLOGY

II.1. Systematic Review of Situational Analysis of Irrational use, Presence and Extend of Substandard/ Falsified/ Counterfeit Medicines

II.1.1. Literature search strategy and study selection

We searched MEDLINE (via PubMed) and Google scholar for articles published until December 2019, without any language restriction. The combinations of the following search terms were used: "Analysis" or "investigation" or "study", "Irrational use", "Substandard medicines" or "Falsified medicines" or "Counterfeit medicines", "Substandard drugs" or "Falsified drugs" or "Counterfeit drugs", "Substandard antimalarial drugs" or "Falsified antimalarial drugs " or "Counterfeit antimalarial drugs ", "resistance" or "treatment failure". The search was performed independently by two investigators who identified articles in sequential fashion (titles, abstracts, and then full texts). In addition, references cited in the selected articles and published reviews were manually searched in order to identify any additional relevant studies. Any disagreements were resolved by consensus or review by a third investigator. Article was included if it reported data about the situational analysis of irrational use, presence and extent of substandard/ falsified/ counterfeit medicines. Case-control, cohort and cross-sectional studies were eligible for inclusion. During the screening steps, we excluded animal studies, review articles, reports, editorials and comments.

II.1.2. Data extraction, assessment, and synthesis

Two reviewers independently extracted data from included studies on settings, design, populations' and characteristics. Disagreements were settled by consensus between the authors. The following data were extracted from each eligible article: author's name, year of publication and data related. The STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) checklist was used for quality assessment. Briefly, we assessed the quality of included studies in accordance with the following criteria: study design, selection criteria and basic characteristics of study participants, duration of treatment, methods and its reliability. **But, due to the heterogeneity in the methodology used and in data presentation, the research team decided to select and comment some interesting published studies that**

emphasized the irrational use of medicines and its impact, by the possible presence of fake antimalarials in the results chapter of the Thesis.

II.2. Pharmaceutical quality of some Artesunate Amodiaquine combinaisons in Cameroon

II.2.1. Study Design

This part was an experimental and cross sectional study.
This study was carried out at the National Drug Quality Control and Valuation Laboratory/ Laboratoire National de Contrôle de Qualité et d'Expertise (LANACOME).

II.2.2. Duration of Study

This part was carried out over a period of 5 months, from January to May 2017, in Yaoundé.

II2.3. Origin and sampling

Consecutive sampling was done in order to obtain 15 batches of different dosages (25/67,5mg, 50/135mg and 100/270mg), infants, children, adolescents and adults of the artesunate-amodiaquine combination. Sampling was done only in Cameroon's legal procurement system, which comprises two sectors: the public and the private sector. In the public sector, samples were collected in hospitals, health centers and the regional medicine stores. In the private sector, samples were collected from community pharmacies and wholesalers. For each sample, a detailed form was filled in with: the name of the specialty or generic under which the product is marketed, the international nonproprietary name, the lot number, the dosage, the laboratory manufacturer, the quantity, the date of manufacture, the expiry date, the city of sampling, the place of sampling, its origin of manufacture. We also checked if these specialties have a Market Authorization in Cameroon.

Inclusion Criteria

- ACT's tablets of Artesunate Amodiaquine,
- found in agreed wholesailers stores, hospitals, health centres and regional medicine store.

Non Inclusion Criteria

- Tablets of artesunate-amodiaquin from illicit market and other galenical dosage forms.

II.2.4. Pre analytical preparation

***Laboratory Reagents*:** Acetonitrile R for HPLC; Anhydrous dihydrogen Phosphate; Sodium Octanesulfonate R; Methanol; Ammoniac; Water; Phosphoric Acid; Hydrochloride Amodiaquine Chemical (*Reference Substance/ Certificate of Analysis (Batch Number : 1921160), Réf stockholm, sweden, SN : 2371035 EDQM*), Artesunate Chemical *Reference Substance Certificate of Analysis. (Batch Number: 45422), CS30025-F67081strasburg (France) EDQM*).

***Laboratory Material*:** Test Tubes; Pestle and Mortal; volumetric Pipettes (*2ml, 3ml*); Volumetric flask (*20ml, 25ml, 50ml, 100ml*); Graduated cylinders; Micropipettor; Plate: Silica gel; Electronic balance (*METLER TOLEDO brand SANTRUIS with a precision to 1 / 10th of a milligram*); UV lamp (254nm, 366nm); Spatulas; vortex; UV-visible spectrophotometer (*Lambda 25, Serial number 501S10072802, Manufacturer: Perkin Elmer.LAN 064*); HPLC with Agilent Technologies Model (*Infinity 1260 Series iodine array detector, series DEAEQ05576*); Disintegration apparatus (*brandERWEKA, model ZT3*); Dissolutest unit (*brandSotax*); Friability Test (*brand Sotax, model FT2*); Ultrasonic baths; Filter paper; 10ml syringes. The glassware used was that of class A. The choice of the elements used was made according to the necessary precision (volumetric flask, pipette with 2 lines). The weighings were made on a precision scale (at $1/10^{th}$ of a milligram). The use of ultrasound to dissolve the reference substances was necessary.

Study method

The various drugs were firstly sampled. Physical (for labeling control) and pharmacotechnical tests were carried out, followed by the identification of the two active compounds by the TLC method as well as the dissolution of Amodiaquine; and ending with the physicochemical testing. On the other hand, the identification of those two active compounds were also done by the HPLC method as described in the dose control protocol developed and validated in ANSM's laboratories.

Reference substances

Reference substances required for the study were: *artesunate and hydrochloride of Amodiaquine dehydrate, each having a certificate of analysis with the characteristics of the*

molecule, the water content of each and other important parameters for the 'analysis. Reference substances and samples were stored away from light and moisture. Reference substances were ideally stored at 2 and - 80°C; the temperature was routinely checked and taken every day morning and evening.

II.2.5. Physical tests

II.2.5.1. Presentation of the sample: primary packaging

The primary packaging was performed according to LEEM standards. The mandatory information such as *the INN, the manufacturer's name, the date of manufacture, the expiry date, the batch number, the dosage and the pharmaceutical form were verified to be present on the immediate packaging.*
Samples were kept in LANACOME sample library at a temperature between 4 and 8°C .

II.2.5.2. External packaging

This has been assessed in accordance with the requirements of the Marketing Authorization requirements in Cameroon. The first marketing authorization, an information database in the two mandatory languages (English and French) of these drugs in Cameroon were checked.

II.2.5.3. Organoleptic characters

The shape, color, taste and type of tablets (simple or coated) were identified and a sample of 10 tablets randomly selected, according to European Pharmacopoeia, 9^{th} Edition, Vol.1 specifications. The quality of these tablets were also checked: cracked, broken or with signs of degradation.

II.2.6. Pharmacotechnical tests

These testings were done according to European Pharmacopoea, Edition 9, Volume. 1

II.2.6.1. Uniformity of mass

Twenty tablets per batch, were randomly selected and weighed, from the control sample and their average mass determined according to the standards of the European Pharmacopoeia, Edition 9, Volume 1, 2017.

Operating conditions: Electric scale: SARTORIUS brand, model X104 with a precision of 1 / 10^{th} of a milligram.

II.2.6.2. Disintegration

The disintegration test was carried out as follows:

In each of the 6 tubes of the apparatus, a tablet was introduced followed by a disc; they were then placed in the cylindrical vessel, containing distilled water at 37 ± 2 ° C. The device was ran and the time at which the tablets were totally broken down were recorded. The Standard established by the European Pharmacopoeia Edition 9, Volume 1 stated that: time should be ≤ 15 minutes.

Operating conditions

Equipment used: disintegrating device: brand ERWEKA, model ZT3, Disk,Temperature 36.50C. Disintegration chamber containing 800ml of distilled water.

II.2.6.3. Friability

To perform this test, according to European Pharmacopoea, 20 tablets were weighed per batch:

- For the unit mass of ≤ 0.65g, the friability test must be done on a number of tablets equivalent to 6.50g; therefore, the friability test was done on 10 tablets. The Friability Test device was set at 100 rotations in 4 minutes; the device was then turned on, tablets collected, dust was eliminated, tablets weighed, and placed inside the drum, removed and tablets weighed again.

"*The maximum loss should be less than 1 percent as described by the European Pharmacopoeia Edition 9 Volume 1*".

The Equipment used was a brand Sotax mode lFT2.

II.2.7. Physicochemical tests

II.2.7.1 Dissolution

The Dissolution Test was carried out with distilled water; for the dosage, the USP Pharmacopoeia 2017 TOME 2 method, was used as: *Medium: water 900ml, Device 2: 50 rpm, Time: 30minutes, UV detector: 342 nm,* STANDARD SOLUTION used was USP Amodiaquine hydrochloride RS. The **Sample Solution was obtained by** filter the parts of the solution under test, suitably diluted with water, if necessary in comparison with USP

Amodiaquine hydrochloride RS. The **Reagents were** standard solution and sample solution; then the amount of Amodiaquine hydrochloride was determine.

Tolerance: the equivalent amount of Amodiaquine hydrochloride should not be less than 75%.

Water was introduced in the tank to the required limit, then 6 vases were filled with 900ml of distilled water, and put on the device. When the temperature of 37 ±0.5°C was reached, 1 tablet was introduced in each of the 6 vases. At 30 minutes' time indicated by the protocol. The device was stopped and in an interval of <> 2% time, the required quantities were taken for the assay by spectrophotometer, at the various dilutions, and results were noted.

Operating conditions

The Dissolution apparatus: brandSotax, Temperature: 37 ± 0.5°C.

II.2.7.2. Identification of artesunate and Amodiaquine by Thin Layer Chromatography

Thin layer chromatography uses a thin glass plate coated with either aluminum oxide or silica gel as the solid phase. The mobile phase is a solvent chosen according to the properties of the components in the mixture. The method of identification of artesunate and Amodiaquine was that given by the MINILAB kit in which was found a method of identification of antimalarial combinations.

Principle

The principle of TLC is the distribution of a compound between a solid fixed phase applied to a glass or plastic plate and a liquid mobile phase, which is moving over the solid phase. A small amount of a compound or mixture is applied to a starting point just above the bottom of TLC plate. The plate is then developed in the developing chamber that has a shallow pool of solvent just below the level at which the sample was applied. The solvent is drawn up through the particles on the plate through capillary action, and as the solvent moves over the mixture each compound will either remain with the solid phase or dissolve in the solvent and move up the plate. Whether the compound moves up the plate or stays behind depend on the physical properties of that individual compound and thus depend on its molecular structure, especially functional groups. The solubility rule "Like Dissolves Like" is followed. The more similar the physical properties of the compound to the mobile phase, the longer it will stay in the mobile phase. The mobile phase will carry the most soluble compounds the furthest up the TLC plate.

The compounds that are less soluble in the mobile phase and have a higher affinity to the particles on the TLC plate will stay behind (Singhal *et al.*, 2009).

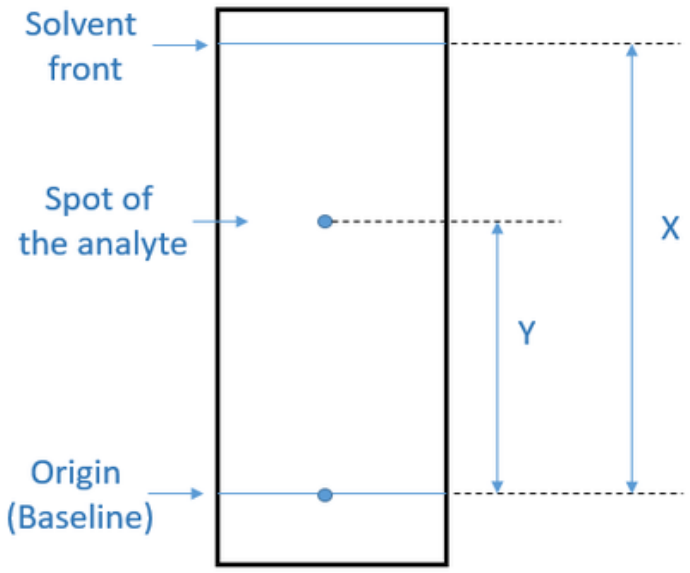


Figure 8: Measurement of Retention Factor (Rf) after TLC plate development

B / A = Rf

B = distance travel by substance (B), A = distance travel by the solvent front (A), TLC presents advantages as follows: Rapid implementation (1 à 2 hours), Simplicity – Affordability – Sensitivity (ug)

Preparation of Standard Solution and Sample for Amodiaquine

After grounding the tablets in a mortar, the required quantities associated with each assay were weighed to be able to have a weight of 0.625mg at the end of the analysis. This weighed mass was dissolved in 10 ml of distilled water. One ml of this solution was collected and added to 10 ml of methanol using pipette.

Development and detection for Amodiaquine

Using a pipette, 5 ml of ethyl acetate, 20 ml of methanol and 0.5 ml of concentrated ammonia solution were introduced in the chromatographic tank. The tank was then closed in other to avoid saturation of the medium for 15 minutes. During this time, spots of the different samples and reference standard were deposited on the chromatographic plate. Subsequently, it was dried using a dryer and introduced into the tank. It was then allowed to migrate for 20

minutes and the plate removed from the tank and the solvent front marked. It was allowed to dry at room temperature. The chromatographic plate was revealed with the UV lamp at 254 nm.

Preparation of the Standard Solution and Sample for *Artesunate*

After pulverizing the tablets in a mortar, the required quantities associated with each assay were weighed. This weighed mass was dissolved in 20 ml of methanol.

Development of the Chromatogram and revelation of spots for Artesunate

Using a pipette, 18 ml of ethyl acetate, 4 ml of acetone and exactly 0.1 ml of anhydrous acetic acid were introduced in the chromatographic tank. The tank was then closed to avoid saturation of the medium during 15 minutes. The spots of the different samples and reference standard were then made on the chromatographic plate. Subsequently, it was dried using a dryer and introduced in the tank. It was allowed to migrate for 10 minutes and the plate removed from the tank and the solvent front marked. It was allowed to dry at room temperature. The revelation of artesunate was made with sulfuric acid and methanol. For this, a 250 ml beaker was filled with 190 ml of methanol and 10 ml of concentrated sulfuric acid solution. The mixture was stirred and allowed to cool. Subsequently, the mixture was sprayed on the plate and the excess liquid drained. The plate was allowed to dry on a hot plate and after a time of warming, the artesunate stains appeared gradually in the dray light.

II.2.7.3. Determination of the two active ingredients by HPLC

The quantitative analysis of artesunate and Amodiaquine was performed by High Performance Liquid Chromatography using the method developed by ANSM (French National Agency for the Safety of Medicines). This method makes it possible to elute the two active ingredients simultaneously).

Chromatographic parameters

COLUMN: C18 grafted silica type, 100 mm long and 4.6 mm internal diameter, with a particle size of 3 µm. The Hypersil BDS model is suitable. **Mobile phase A:** 1,36g of anhydrous dihydrogen phosphate and 21.6 g of sodium octanesulphonate were dissolved in R water, and made up to 1000 ml with the same solvent (water). Then the pH was adjusted to 3.0 with phosphoric acid R. The mixture was finally filtered to avoid damaging the column

which is very sensitive. **Mobile phase B:** acetonitrile R for HPLC. Flow rate: 0.8 ml / min; Column temperature: 20°C; Detection: spectrophotometer at 210 nm for artesunate, at 300 nm for Amodiaquine; Injection volume: 10 µl; Autosampler temperature: refrigerated; Recording time: 15 minutes; Compliance solution and system stabilization: a stabilization period was rigorously respected. No tests were performed until the system's compliance criteria were met. In these conditions, *the retention time of Amodiaquine was about 4 minutes, that of artesunate about 10 minutes.*

Sample solution: 25.0mg of artesunate and 88.0mg of hydrated Amodiaquine di hydrochloride (corresponding to 67.4mg of Amodiaquine base) were dissolved in the dilution solvent, and then the solution was sonicated for 5 minutes. Subsequently 2 solutions per test: T1, T2 were prepared. **Note**: The conversion factor of Amodiaquine dihydrate hydrochloride to Amodiaquine base is 355.9 / 464.8 = 0.766 with (*355.9 = molecular weight of amodiaquine base and 464.8 = molecular weight of amodiaquine dihydrate hydrochloride*).

Principle of High Pressure Liquid Chromatography

High Pressure Liquid Chromatography (HPLC) is a method of separation based on the analyte's relative solubility between two liquid phases. HPLC utilizes different types of stationary phase (typically, hydrophobic saturated carbon chains), a pump that moves the mobile phase (s) and analyte through the column, and a detector that provides a characteristic retention time for the analyte. Analyte retention time varies depending on the temperature of the column, the ratio/composition of solvent (s) used, and the flow rate of the mobile phase. With HPLC, a pump (rather than gravity) provides the higher pressure required to propel the mobile phase and analyte through the densely packed column (Figure 9).

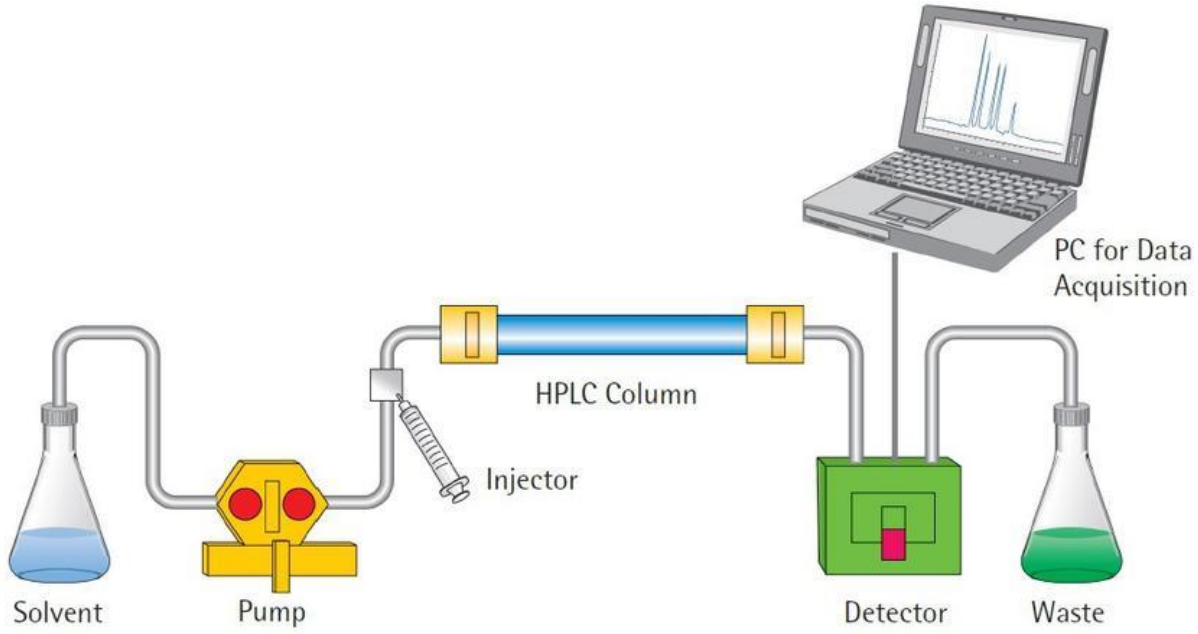


Figure 9: Block diagram showing the components of an HPLC instrument

HPLC is a technique for separating compounds in solution. Upstream of the HPLC analysis itself, various steps take place:

- Preparation of one or more mobile phases
- Preparing the diluent
- Mounting the column in the column oven
- Program the method (detection wavelength, furnace temperature, flow rate, analysis time, injection volume, type of elution)
- Column equilibration which involves passing the mobile phase through the column at the rate of the analytical method in order to stabilize the baseline.
- Create the analysis sequence which is the order of injection of the different solutions (blank, control solutions, sample test solution)

Preparation of standard solutions and test

Data processing

The solutions prepared are as follows:

- Two control solutions of the same concentration
- Three test solutions per sample of the same concentration as the reference solutions
- A blank which is the dilution solution for samples and controls.

During the analysis, one of the test controls is injected six times. This is to check the conformity of the system. From where you have to calculate the coefficient of Variation (CV) of these six injections:

CV = (Standard deviation of the areas obtained) / (average of the areas obtained) X100 With CV≤1.0%

The second test control is injected twice.

After injection of the controls, the Recovery Factor is calculated: to ensure the correct preparation of the standards.
The overlap factor between controls is calculated as follows:

Fr = (Area std1) / (Area std2) X (mass sdt2) / (mass std1) X100
with 98.0% ≤Fr≤102.0%

After injection of the controls, the test solutions of the samples are injected in a well-defined sequence.
From the areas obtained, the concentrations of the test solutions are calculated, according to the formula:
T (mg) = (Test area) / (Std area) X (std mass) / (test mass) X (std title) / 100 X (Test dilution volume) / (std dilution volume) X Average weight

Then, we calculate the average of these concentrations obtained and the CV (≤2.41%) of these concentrations. The calculated mean and CV values are compared to the specifications.
The results obtained will lead to a decision making.

Dosage

For 1000 ml of solvent, 1.36 g of anhydrous hydrogen phosphate was weighed and added to 1000 ml of water R. Then the pH was adjusted to 3.0 with phosphoric acid R. Sixty volumes of this solution were then mixed with 40 volumes of acetonitrile R. Five tablets of Artesunate-Amodiaquine 25 / 67.5 were homogenously crushed. The test portion was then dissolved in the dilution solvent. The solution was then passed with ultrasound for 5 minutes, homogenized 1 minute by vortex, and supplemented with 50.0 ml with the same solvent. For each batch three solutions E1, E2 and E3 were prepared from the same grind. For the other dosages, the calculations were reduced to the same concentration.

II.3. Pharmacoeconomics of substandard/ falsified/ counterfeit antimalarials: public health and economic impact on development

We search reference articles on pharmacoeconomics of substandard /falsified/counterfeit antimalarials, and based on databases related to articles generated by:

i. The study on the public health and socioeconomic impact of substandard and falsified medical products (by the state members of the WHO) in 2015; aimed be an advocacy tool to highlight the scope and scale of the problem of substandard and falsified medical products and the harm they cause,

ii. A systematic review and meta-analysis by Sachiko Ozawa and collaborators (2017) in which five databases: PubMed, EconLit, Global Health, Embase, and Scopus were searched from inception until November 3, 2017. Before inclusion in this systematic review, publications were assessed to determine whether they examined medicine quality and the prevalence and/or economic burden of substandard and falsified medicines in low and middle-income countries and

iii. The study by Nafiu and collaborators, (2017) on Unveiling the peril of substandard and falsified medicines to public health and safety in Africa.

II.4. Study design of efficacy of SP+AQ *vs* AS+AQ in Northern regions

II.4.1. Description of the Study Sites

The study was conducted at the Garoua Regional Hospital, in the North region and the Maroua Regional Hospital (Far North Regions), two geographically distinct sites. These sites fall within the major Sahelian geo-ecological zoneof Cameroon (Kleinschmidt *et al.,* 2001). Garoua in the North Region of Cameroon and located at coordinates 6º24'N & 10º46'E. Garoua serves as a river port in years with abundant rainfall. Situated in the River Benue Basin, it receives an average annual rainfall of 380 mm with just about 4 months of rainy season. It was the town in which Mefloquine resistance was reported to be highest in the country by the end of 1985 (Brasseur *et al.,* 1988). The average temperature is 31˚C for most of the year and the vegetation is Guinea-Savanna (Kleinschmidt *et al.,* 2001). The population is predominantly Muslim and is comprised mainly of livestock farmers. Some, however, have begun trading with neighboring Nigeria. Maroua is in the Far North Region of Cameroon and located at coordinates 5°09′ N, 12°27′E. Mount Maroua dominates the skyline of the city. The climate of Maroua is Sahelian, hot and dry for most of the year. Maroua is along the same belt stretching from the West African country of Gambia through Burkina Faso and Niger to Northern Nigeria and across Chad and towards Sudan. This region experiences low precipitation, and therefore is prone to malaria epidemics, with the most recent one in 2013. The area has recently suffered from flash floods that resulted in the displacement of communities and unprecedented surges in infections such as malaria. The predominant religion is Islam and most engage in agriculture (cotton farms) or raise cattle (Non-Fulani and Fulani respectively). They also trade with neighboring Chad and Nigeria.

II.4.2. Epidemiology description

The North region is located in the area of dry savannah and is characterized by a rainy season of 3 to 5 months with annual rainfall reaching 1000 mm / year. According to the malaria stratification, this area belongs to a hyper endemic malaria stratum with seasonal transmission of the malaria parasite prone to cyclical outbreaks (the transmission period could be extended compared to the Far North region). The population of the area is estimated at 2.4 million inhabitants over an area of 66,090 km^2.

Before the intensification of Long-lasting insecticidal nets (LLINs) in the country, the prevalence of the malaria parasite in children aged 1 to 15 ranged between 6.5 and 30.7% (n = 655) in cross-sectional surveys in the Lagdo health districts (*Atangana J. et al., 2010; Atangana J. et al., 2012; Atangana J. et al., 2012*).

The entomological inoculation rate (EIR) was found to vary between 2.7 and 36.5 infected bites / person / month (*Robert V. et al, 1992, Antonio-Nkondjio C. et al, 2008*). After the intensification of LLINs, average malaria parasite prevalence levels of 30.4% [varying considerably between 28.6% (798/2795) for net users and 35% (243/694) for non-users. net] were recorded in the health districts of Garoua, Pitoa and Mayo-Oulo in children aged 6 months to 5 years (Table 2) (*Lancet Infect Dis. 2018*). Intense transmission occurred during the rainy season with estimates varying from 24.5 to 60 infectious bites / person / month in the health districts of Lagdo, Garoua, Pitoa, Mayo Mbocki and Mayo Oulo (*Robert V. et al., 1992; Antonio-Nkondjio C. et al., 2008 ;Tabue RN., et al.,2017; Awono-Ambene PH, et al., 2018*). The increase in the EIR rate recorded for this region could be due to the fact that entomological surveys were undertaken at different sites before and after the scale-up of LLINs. It is possible that localities with a high EIR had a much higher rate before LLIN scale-up. The main vector species in the area are An. Arabiensis, An. Gambiae and An. Funestus. Other species involved in the transmission of malaria parasites are An. Pharoensis, An. Coluzzii, An. Rufipes and An. Ziemanni (*Antonio-Nkondjio C. et al., 2008; Tabue RN. et al, 2017*).

II.4.3. Target Population and Duration of Subject Participation

235 Children of either gender, between 6 months and 10 years of age, with acute uncomplicated *P. falciparum* malaria, or who fulfil all of the inclusion and have none of the exclusion criteria were recruited from the outpatient clinics of participating health facilities. The study population was selected from malaria positive patients during routine practice at the study sites.

The duration of each subject participation on completion of the study was 28 days, with each participant required to report to the study clinic on days 1, 2, 3, 7, 14 and 28 or at any other time when clinical sign(s)/symptom(s) of malaria was suspected (WHO, 2009).

II.4.3. Inclusion Criteria

The criteria for inclusion of participants for the study were as follows:

- Children of both genders, aged between 6 months (≥ 5kg) and 10 years.
- Children suffering from acute uncomplicated *P. falciparum* malaria confirmed rapid diagnostic test (Lactate dehydrogenate based).
- Children having fever (axillary temperature ≥ 37.5^{o}C), or a history of fever in the previous 24 hours.
- Children able to ingest oral tablets (either suspended in water or uncrushed with food).
- Willingness to participate in the study with written assent from parent or guardian. Parental authorization was obtained for children less than 18 years old and documented assent of parents/guardians for children 8-10 years.
- Willingness and ability to attend the clinic on stipulated regular follow-up visits.

II.4.4. Non Inclusion Criteria – Exclusion criteria

A child was not included from being enrolled to the study if any of the following "danger signs of severe malaria": were observed:

- Not able to drink or breast feed.
- Persistent vomiting (>2 episodes within the last 24 hours)
- Seizures (>1episode within the last 24 hours).
- Lethargic/unconscious; signs or symptoms, indicating severe/complicated malaria according to WHO criteria (WHO definition).
- Concomitant illnesses, underlying chronic hepatic or renal disease, abnormal cardiac rhythm, hypoglycaemia, jaundice, respiratory distress
- Serious gastro intestinal disease, severe malnutrition (W/H < 70%) or severe anaemia (haemoglobin < 5 g/dl).
- Known hypersensitivity to SP+AQ, AS+AQ and other drugsused in the study.

II.4.5. Sampling Technique

Consecutive sampling was applied and each study participant was randomised to one of the 2 trial arms, i.e., Study Arm-A: Artesunate-Amodiaquine (AS+AQ); Study Arm-B: Sulfadoxine/ Pyrimethamine-Amodiaquine (SP+AQ) in the ratio of 1:1.

To determine the efficacy of SPAQ, at a significance level of 5%, a power of 80%, and a precision of 5%, the sample size (n) was given by*(Aday & Cornelius, 2006)*:

$$\mathbf{n= (1.96)^2P[1-P]/d^2}$$

***d**, the precision desired in the efficacy rate; **P,** the best estimate of what the cure rate might be.*

In this case, a cure rate of 82% as reported by a previous study in Cameroon was used *(Mbacham et al., 2010*). Therefore, **n= 227** participants were needed. But assuming a loss to follow up rate of 10% from previous studies, a total of **227+22.7** = **250 participants** was the target. Given two sites (North and Far North) and two arms per site, each site had to recruit 63 patients in the AS+AQ arm and 63 in the SP+AQ arm.

II.4.6. Clinical Assessment

II.4.6.1. Preparatory Phase and Initiation of the Study

On confirmation of financing from the Global Fund to Fight AIDS, Tuberculosis and Malaria and coordination from the National Malaria Control Programme, the development of the project proposal, as well as requests for the acquisition of ethical clearance from the Cameroon National Ethics Committee (NEC) was launched. Few exchanges occurred between the NEC and the study team before issuing of the ethical clearance. Administrative authorizations were obtained from the North and Far North Regional Public Health Delegates. Once the authorizations were obtained, case report forms (CRFs), a field manual, standard operating procedures (SOPs) for all clinical and laboratory activities related to the study were developed and validated by the principal investigator. After a series of team meetings and a clear understanding of the procedures, roles were assigned to each team member based on their competence. Finally, on approval by all administrative authorities, a team of 3 investigators (2 medical doctors and a biochemist) made a visit to the North and Far North Regions to initiate the study as recommended in a recent study (Mbacham *et al.,* 2014). The team carried out a 3-day training session per region, and activities included: presentation of the study and training of 7 medical doctors, 7 nurses and 7 laboratory technicians on the procedures of the trial and their responsibilities. The three-day training focused on the administration of informed consent (Appendix 7); how to identify signs and symptoms of malaria; safety assessment and adverse event reporting, recording of data, and so on. The

training was done with the help of power point slides and a role play performed by the trainees. The trainees all signed an attendance sheet to mark effective presence.

II.4.6.2. Ethical Consideration

Ethical clearance N° 2015/03/567/CE/CNERSH/SP was obtained from the Cameroon National Ethics Committee (Appendix 1). Authorization letters to carry out the trainings and to perform the study were granted by the Regional Delegates of Public Health of the North and Far North Regions. **Informed Assent Forms** (Appendix 2) were signed by parents of children (6 months to 10 years) with acute malaria infection to take part in the study

II.4.6.3. Procedure (Treatment and Follow Up)

In conformity with routine malaria diagnosis at the study sites, the same flow of patients was maintained. As recommended by the National Malaria Control Program, the first line of treatment of simple, uncomplicated malaria is Artesunate + Amodiaquine (AS+AQ), reason why AS+AQ was used as the comparator drug in this trial.

Day 0 (Screening, Enrollment and Randomisation)

On the first day, parents/guardians of children presenting with acute uncomplicated falciparum malaria were approached, informed verbally as well as in writing about the nature of the study, the anticipated risks and benefits, the right to interrupt their participation at any time. Informed assents were then obtained from these parents/guardians (signatures or thumb impressions) on acceptance to participate in the study (Appendix 2). After signing the assent, a rapid diagnostic test (RDT) was the first test to be carried out on the study participants as recommended by the National Malaria Control Programme (NMCP) of Cameroon *(PNLP, 2014)*. When the RDT was positive for malaria, the patients had to meet all the inclusion criteria and none of the exclusion criteria. The patients were then randomised to one of the drug arms (AS+AQ or SP+AQ) and given the first day of age-specific antimalarial treatment as recommended by WHO (Appendix 3). Patients underwent clinical and laboratory assessments: *blood smears (thick and thin blood films) for microscopy; dot blood spot on filter paper for molecular analysis; serum hemoglobin; and blood glucose levels*. Data on complete medical history, clinical and laboratory evaluations were collected and recorded in their respective CRFs (Appendix 4). Patients were subsequently requested to come back for follow up and at any time when sign(s)/symptom(s) of malaria was suspected. If the

participant did not come to the hospital on the expected visit date, they were eventually visited at their homes. The table below presents the implementation of the study on randomised participants.

Table 3: Presentation of the study on randomized participants

Period	Clinical Examination	Antimalarial Treatment administred	Laboratory assessment	ObservationsRecommendations
Day 0	**Screening, Enrolment and Randomization**			
Day 1 Day 2	Complete: *clinical symptoms of malaria* *Medicines anamnesis*	Second dose Third dose	Blood smear for microscopy	At the end of treatment on Day 2, patients encouraged to return the following day for follow-up
Day 3	Assessing clinical symptoms of malaria; and taking a history of concurrent medication.	Completed a 3-day antimalarial treatment (Days 0, 1 and 2).	blood smear for microscopy	If no adverse events reported, participant was given rdv for the 7th day. If they missed or could not make it on day 7, they could return a day before or a day after as their visit of Day 7.
Day 7	Complete clinical examination; assessing clinical symptoms of malaria; and taking a history of concurrent medication	No medications given	Blood stain on the filter paper for molecular analysis, blood smear for microscopy and serum hemoglobin.	With no reports of adverse events the participant was said to have successfully completed the study. If they missed or could not make it on day 14, they could return a day before or a day after as their visit of Day 14.
Day 14	Complete clinical examination; assessing clinical symptoms of malaria; and taking a history of concurrent medication.	No medications given	Blood smear for microscopy only.	With no reports of adverse events the participants were asked to return on day 28. If they missed or could not make it on day 28, they could return 2 days before or after the set date and that visit would be considered as their Day 28 visit.
Day 28	last day of the study for the participant Complete clinical examination; assessing clinical symptoms of malaria; and taking a history of concurrent medication.	No medications given	Blood smear for microscopy and serum haemoglobin	. With no reports of adverse events the participant was said to have successfully completed the study. The evaluation parameter on this day was: Efficacy, which consisted of: ***parasitological cure rates adjusted by PCR at day 28; Prevalence of Early Treatment Failure (ETF), Late Clinical Failure (LCF), Late Parasitological Failure (LPF), Adequate Clinical and Parasitological Response (ACPR) as recommended by WHO***

II.4.6.4. Adverse Event and Severe Adverse Event Reporting

On visiting days or any other day of the study, parents/guardians were advised to report to the hospital on any adverse event which may have occurred to the participant. The parameter of evaluation was Safety, which consisted in: *Prevalence of adverse events and severe adverse events occurred following drug administration. This AE or SAE was graded according to the NIH Common Toxicity Criteria* (Appendix 5). Participant's management was immediate and consisted in relieving the patient from the symptoms. The decision to exclude a participant from the study due to adverse events depended on the severity of the events.

II.4.6.5. Storage and Transportation of Samples

The slides were grouped per participant, labelled accordingly and placed in a slide box. The RDTs were labelled as per participant and stored in sealed envelopes. The filter papers were labelled and stored with silica gel in separate envelopes for each participant. The envelopes were labelled as well. At the end of the study, all sealed samples were packaged in separate trunks (North and Far North regions) and transported at room temperature to the Biotechnology Centre, University of Yaoundé I, where they were further analysed.

Table 4: Tabular Summary of 28 Day Study Participation

Activity	**D0**	**D1**	**D2**	**D3**	**D7 +/- 1day**	**D14 +/- 1day**	**D28 +/- 2days**	**AE or SAE**
WrittenParentalAssent	✓							
Inclusion/Exclusion Criteria	✓							
Demography&MedicalHistory	✓							
RDT	✓							
Complete Clinical Examination including PE, Vital Signs, Axillary Temperature	✓	✓	✓	✓	✓	✓	✓	✓
Assessment of Clinical Symptoms of Malaria	✓	✓	✓	✓	✓	✓	✓	✓
Concurrent Medication	✓	✓	✓	✓	✓	✓	✓	✓
Randomisation	✓							
AS+AQ or SP+AQ Administration	✓	✓	✓					
Microscopy	✓	✓	✓	✓	✓	✓	✓	✓
Blood sample for parasite DNA (filter paper)	✓				✓			✓
SerumHemoglobin	✓				✓			
Blood Glucose	✓							

II.4.7. Molecular Analysis

II.4.7.1: DNA Extraction by the Chelex method

Principle

This method is based on the disruption of the cell membrane in the presence of saponin. Proteins and other molecules are washed out by Phosphate Buffered Saline (PBS). When heated, the Chelex which is a cationic resin, chelates all cations, and leaves the negatively charged DNA in solution (Plowe *et al*., 1995).

Experimental procedure

Before the procedure, all materials used were sterilized in the autoclave then the working surface sterilized with ethanol-70% and bleach-10%. The blood spots were excised, transferred into Eppendorf tubes (1.5 ml), and 1ml of saponine 0.5% in 1× Phosphate buffer saline (PBS) was added for cellular lysis. The tubes were inversed several times and incubated at 4°C all night. The scissors were flamed, cooled in double distilled water (ddH_2O) and dried with tissue paper after each excision. The following day, the brown solution was thrown from the tubes and the blood spot washed with 1ml 1×PBS at 4°C for 15-30minutes. After the supernatant was discarded and the filter papers transferred to Eppendorf tubes (1.5 ml) containing 50 µl of Chelex-100 at 20% and 150µl of DNAse free water. The mixture was then placed into a heater block at 100°C. The forceps were sterilized after manipulation of each sample. After 10 minutes, we mixed the solution vigorously using a vortex for 30s. This action was repeated twice per tube. The tubes were centrifuged at 10,000 rpm for 2minutes, and then we pipetted 150µl of the supernatant and introduced into new tubes. The centrifugation was repeated againat 10,000 rpm for 2 minutes, the 100µl of the supernatant was pipetted one last time avoiding the chelex resin, and put into new tubes. The solution was then conserved at - 20°C.

II.4.7.2. Genotyping of Malaria Parasites

To confirm treatment failure from the cases declared through microscopy, and differentiate between recrudescence and re-infections from the confirmed treatment failure cases, the merozoite surface protein-2 (*msp2)* gene was amplified in a two round PCR (outer and inner) procedure as described (Cattamanchi *et al.,* 2003). For each treatment failure confirmed through microscopy, the DNA extracts for Day 0 (pre-treatment) and Day 7(post-treatment)

were amplified and the molecular detection of the msp2 gene compared between the two days for each sample.

Principle of PCR amplification

The PCR is an *in vitro* amplification method used to enzymatically synthesize defined DNA sequences. The reaction uses two oligonucleotide primers that hybridize to opposite strands and flank the target DNA sequence to be amplified. Primer elongation is catalyzed by the thermostable Taq DNA polymerase. Repetitive series of cycles involving template denaturation, primer annealing and extension of the annealed primers by Taq DNA polymerase result in an accumulation of specific DNA fragments.

For the outer msp2 gene amplification, the reaction mix for each sample consisted of:

- 18.25µl of NFW
- 2.5µl of 10 X thermopol buffer
- 0.5µl of 10 mM dNTPs (dATP, dGTP, dCTP, dTTP)
- 0.25µl of 2.5 µM of each primer (S2 and S3)
- 0.25µl of Taq DNA Polymerase (5units/µl); and
- 3µl of parasite DNA extract obtained by Chelex method (Day 0 and Day 3)

This gave a total volume of 25µl per tube. Each tube was placed in a thermocycler to a corresponding programme under the following reaction conditions:

- Pre-denaturation at 94°C for 3mins; then 30 cycles in thermocycler for:
  - Initial denaturation at 94°C for 30sec
  - Hybridization at 42°C for 1min
  - Elongation at 65°C for 2mins
- Final extension at 72°C for 3mins

For the inner *msp2* gene amplification, the reaction mix for each sample was as follows:

- 20.25µl of NFW
- 2.5µl of 10 X thermopol buffer
- 0.5µl of 10 mM dNTPs (dATP, dGTP, dCTP, dTTP)
- 0.25µl of 2.5 µM of each primer (S1 and S4)
- 0.25µl of Taq DNA Polymerase (5units/µl); and
- 1µl of amplicon of the outer msp2

This gave a total volume of 25µl per tube. Each tube was placed in a thermocycler to a corresponding programme under the following reaction conditions:

- Pre-denaturation at 94°C for 3mins; then 30 cycles in thermocycler for:
  - Denaturation at 94°C for 30sec
  - Hybridization at 42°C for 1min
  - Elongation at 65°C for 2mins
- Final extension at 72°C for 3mins

A sample of known species identity was used as the positive control while distilled water was used as the negative control.

Agarose gel electrophoresis of PCR products

To confirm the presence of the msp2 gene in the samples, the final PCR products of the malaria parasites were separated on 2% agarose gel (Seakem, Nusieve).

Principle

This is a technique for the separation, purification and identification of DNA fragments between 0.5-25 Kb. Under an electric field, DNA molecules being negatively charged will migrate in the gel towards the positively charged electrode (anode) with their speed of migration being inversely proportional to their molecular weight. Smaller molecules will move faster than larger molecules. Factors that influence the rate of migration are: the strength of the electrical field, the concentration of agarose in the gel and most importantly, the size of the DNA molecules.

Experimental pocedure

In brief, 2g of agarose powder was weighed into a 250ml Duran bottle with and 100ml of 1 X TBE buffer added. The mixture was allowed to boil on a Bunsen burner and then allowed to cool at room temperature (25˚C) to 60˚C. 2µl of ethidium bromide (mutant agent), was added into the mixture and the bottle swirled slowly to avoid air bubbles. The solution was then poured into a clean, leveled casting tray with combs already inserted and allowed to solidify at room temperature for 45 minutes. On solidification, the combs were carefully removed and the casting tray inserted into the electrophoresis chamber filled with sufficient amount of running buffer (1 X TBE). With a micropipette adjusted to 10µl, the PCR products were gently mixed with 2.5µl of loading buffer and gently loaded into sample wells of the gel.

DNA ladder standard (100bp) was also loaded on the first and last wells at the same time to run simultaneously. The power leads were then connected to the electrophoresis system (Fisher Biotech) set at 75 volts and the system was allowed to run for 40 minutes. The distance migrated was visually monitored by tracking dyes (bromophenol blue and xylene cyanol). The gel was later visualized under a high performance UV transilluminator (UPV) and photographed using a digital camera and the distance migrated calculated against a DNA ladder from a standard curve.

II.4.7.3. Determination of Genetic Polymorphisms in dihydrofolate reductase (*dhfr*) gene

On confirmation of recrudescence from the amplified msp2 samples, PCR was carried out to determine the presence of the folate metabolizing *dhfr* gene in the recrudescent cases. The most common mutations in our context were studied (C59R and S108N). The mutations at codon positions C59R and S108N (nucleic acids: Cysteine [C], Arginine [R], Serine [S], Asparagine [N]) of the dhfr gene were determined by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) as described. The process consisted in amplifying the *dhfr* gene, then digesting the amplification products with restriction enzymes specific to the coding sites mentioned above.

As previously described,

For the outer *dhfr* gene amplification, the reaction mix for each sample consisted of:

- 18.25µl of NFW
- 2.5µl of 10 X thermopol buffer
- 0.5µl of 10 mM dNTPs (dATP, dGTP, dCTP, dTTP)
- 0.25µl of 2.5 µM of each primer (G2 and G3)
- 0.25µl of Taq DNA Polymerase (5units/µl); and
- 3µl of parasite DNA extract obtained by Chelex method (Day 0 only)

This gave a total volume of 25µl per tube. Each tube was placed in a thermocycler to a corresponding programme under the following reaction conditions:

- Pre-denaturation at 94°C for 3mins; then 40 cycles in thermocycler for:
  - Denaturation at 94°C for 1min
  - Hybridization at 50°C for 2mins
  - Elongation at 72°C for 2mins
- Final extension at 72°C for 10mins.

Polymerase Chain Reaction Mixture

	Msp^2	**Dhfr gene**	**CYP2C8 gene**	**NAT2 gene**
NFW(ùl)	18,25 /20.25µl	18,25 /20.25µl	25 µl	18,25 µl
10X Thermopol buffer	2.5 µl / 2.5	2.5 µl / 2.5	2.5 µl	2.5 µl
dNTP(10mM)	0,5 µl of 10Mm/ 0.5	0,5 µl of 10Mm/ 0.5	0,5 µl of 10 mM	0,5 µl of 10 mM
Primer(S2/S1 and S3/S4)	0,25 µl / 0.25	0,25 µl / 0.25	O.8µM	0,25 µl/primer
Taq DNA Polymerase	0,25 µl / 0.25	0,25 µl / 0.25	1.25 U/ µl	0,25 µl
Parasite DNA extract	3 µl / 1 µl of amplicon of outer	3 µl / 1 µl of amplicon of outer	5 µl+	3 µl

Polymerase Chain Reaction work Conditions

Msp^2	**Temperature**	**Duration**	**Nb Cycles**
Pre denaturation	94°C	3 mn	30
Initial denaturation	94°C	30 sec.	30
Hybridization	42°C	1 mn	30
Elongation	65°C	2mns	30
Final extension	72°C	3mns	
Dhfr gene	**Temperature**	**Duration**	**Nb of Cycles**
Pre denaturation	**94°C / 94°C**	**3 mns/ 2mns**	
Initial denaturation	**94°C / 94°C**	**1 mn / 1 mn**	**40/ 35**
Hybridization	**50°C /45°C**	**2 mns / 1 mn**	**40/35**
Elongation	**72°C**	**2 mns / 2 mns**	**40/35**
Final extension	**72°C**	**10 mns / 10 mns**	
CYP2C8 gene			
Pre denaturation	**94°C**		
Initial denaturation	**94°C /**	**5 mns**	**45**
Hybridization	**55°C**	**20 seconds**	**45**
Elongation	**72°C**	**20 seconds**	**45**
Final extension	**72°C**	**3 mns**	**45**

NAT2 gene			
Pre denaturation	**95°C**		
Initial denaturation	**95°C**	**5 mns**	**30**
Hybridization	**55°C**	**50 s**	**30**
Elongation	**72°C**	**50s**	**30**
Final extension	**72°C**	**5 mns**	

For the inner *dhfr* amplification, the reaction mix for each sample was as follows:

- 20.25µl of NFW
- 2.5µl of 10 X thermopol buffer
- 0.5µl of 10 mM dNTPs (dATP, dGTP, dCTP, dTTP)
- 0.25µl of 2.5 µM of each primer (G1 and G4)
- 0.25µl of Taq DNA Polymerase (5units/µl); and
- 1µl of amplicon of the outer dhfr

This gave a total volume of 25µl per tube. Each tube was placed in a thermocycler to a corresponding programme under the following reaction conditions:

- Pre-denaturation at 94°C 2mins; then 35 cycles in thermocycler for:
    - Initial denaturation at 94°C for 1min
    - Hybridization at 45°C for 1min
    - Elongation at 72°C for 2mins
- Final extension at 72°C for 10mins

A sample of known species identity was used as the positive control while distilled water was used as the negative control.

After amplification, RFLP was performed using restriction enzymes for the *dhfr* genes. These restriction enzymes digest the *dhfr* molecule at specific sites defined by a specific number of base sequences called "**restriction sites**". The point mutation on codon 59 involves Cysteine(C) as the wild type and Arginine(R) as the mutant, while the point mutation on codon 108 involves Serine(S) as the wild type and Asparagine(N) as the mutant. Thedigestion enzymesfor the restriction sites of the dhfr gene are ***Xanthomonas manihotis (****Xmn)I* for the C59R mutation and BsrI and AluI for the S108Nmutation. In this study, the *XmnI* and the *Bacillus stearothermophilus (*Bsr)

I enzymes were used.

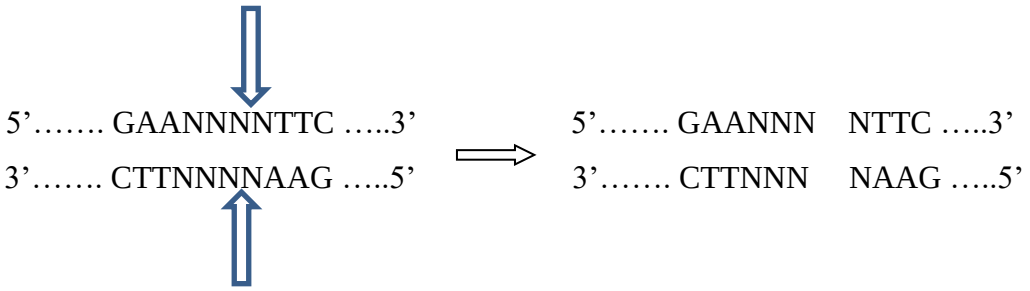
***Xmn I* Digestion**

Xmn I, an enzyme of the bacterium *Xanthomonas manihotis* specifically digests DNA sequences carrying the mutant allele 59Rpolymorphism.The reaction mix for each sample was as follows:

- 10.8µl of NFW
- 2µl of NE Buffer 2
- 0.2µl of Bovine Serum Albumine(BSA)
- 1µl of Xmn I; and
- 6µl of amplicon of the inner *dhfr*

The mixture was then incubated in a thermocycler at 37˚C for 20hours.

The following restriction site is recognized by the enzyme Xmn I after digestion:



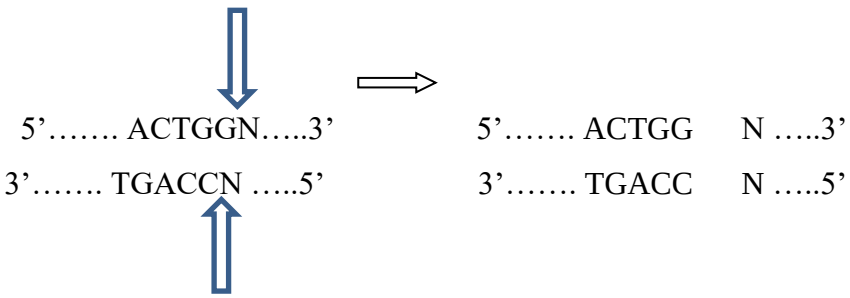
Bsr I Digestion

Bsr I originates from the bacterium *Bacillus stearothermophilus*and digests DNA sequences carrying the mutant allele 108N polymorphism. The reaction mix for each sample was as follows:

- 11µl of NFW
- 2µl of NE Buffer 3
- 1µl of Bsr I; and
- 6µl of amplicon of the inner dhfr

The mixture was then incubated in a thermocycler at 65˚C for 20 hours.

The following restriction site is recognized by the enzyme Bsr I after digestion:



To confirm the presence of mutations on the C59R and S108N codons of the dhfr gene in the samples, the final products of digestion were separated on 2% agarose gel (Seakem, Nusieve) as previously described and the distance migrated calculated against a DNA ladder from a standard curve as described above.

II.4.7.4. Molecular Analysis of CYP2C8 Gene

II.4.7.4.1. Amplification of the CYP2C8 gene

The amplification of the CYP2C8 gene is done according to the adapted approach of Dai and collaborators (2001) and primers (CYP2C8F: 5'-AAGATACATATATCTTATGACATG-3 'and CYP2C8R: 5'-ATCCTTAGTAAATTACAGAAGG-3') were used. For a total of 25 µl, the reaction mixture is composed of PCR water, buffer (10Xthermopol buffer), 10 mM dNTPs (200 µM of each deoxyribonucleotide), 0.8 µM of each primer, 1.25 U / µl of Taq polymerase DNA and 5 µl of DNA extract. The amplification program previously recorded in the thermocycler (T3 thermal cycler (Biometra, UK)) was as follows: the initial denaturation is carried out at 94°C for 5 minutes followed by 45 amplification cycles for which each cycle is a succession of denaturation steps at 94°C in20 seconds, hybridization at 55°C in 20 seconds and extension at 72°C in 20 seconds. The final extension which comes at the end of these cycles is carried out at 72°C. for 3 minutes. At the end of the amplifications, PCR products when not used directly can be stored in the thermocycler at 4°C during 48 hours.

II.4.7.4.2. RFLP digestion of PCR products of the CYP2C8 gene

The restriction enzyme Bcl I was used for digestion of the CYP2C8 gene according to a previously described protocol (Dai et al., 2001). The digestion was carried out in a volume of 20 µl containing PCR water, buffer (10X buffer), 4 U / µl of Bcl I restriction enzyme and 8 µl of CYP2C8 amplicon. This was done in an incubator at 50°C for at least 6 hours (Over night).

II.4.7.5. Amplification of the NAT2 gene

The NAT2 gene was amplified using a T3 thermocycler. The primers that were used to amplify the gene are: NAT-2 (+) 5'-GCCTCAGGTGCCTTGCATTT-3' and NAT-2 (-) 5'-CGTGAGGGTAGAGAGGATAT-3'. Each PCR cycle was performed in a total volume of 25 µl containing: 18.25µl of nuclease free water, 2.5µl of 10Xthermopol buffer, 0.5µl of 10

mMdNTPs, 0.25µl of each primer, 0.25µl of Taq polymerase, and 3µl of the DNA extract. After initial denaturation at 95°C for 5 min, 30 cycles of amplification were carried out with denaturation at 95°C for 50 s, annealing at 55°C for 50 s and extension at 72°C for 50 s, followed by a final extension at 72°C for 5 min.

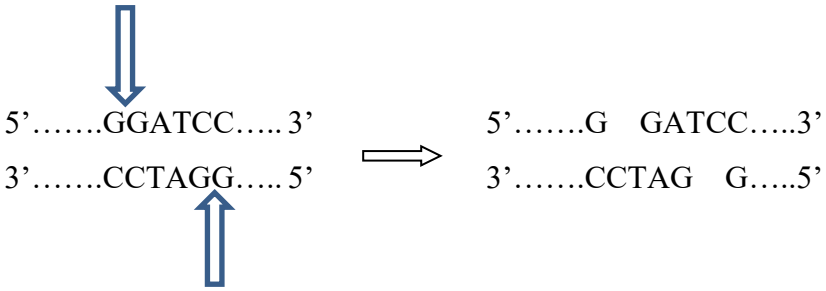
II.2.7.5.1. Restriction fragment length polymorphism (RFLP) of PCR products

After extraction and amplification, the DNA was processed using specific restrictionenzymes which cut the base sequences at restriction sites. Every modification by mutation at restriction sites hindered enzyme action. No enzyme action was then determined by number and length of DNA fragments, obtained after digestion and separation by electrophoresis. The restriction enzymes used to digest the NAT2 gene were: BamH1, Kpn1, Taq1.

II.4.7.5.1.1. BamH1 RFLP digestion

This was done in a total volume of 20 µl which was comprised of: 7.3µl of ddH_2O, 3µl of 10× buffer-3, 0.2µl of BSA 100×, 1.5µl of BamH1 2000 UI/µl and 8µl of the PCR product. The mixture is then incubated at 37°C in a thermocycler for 16hours.

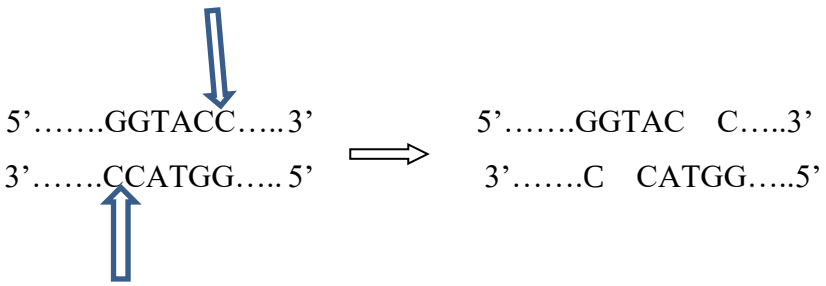
The following restriction site is recognized by the enzyme BamH1 after digestion:



II.4.7.5.1.2. Kpn1RFLP digestion

This was done in a total volume of 20 µl which was comprised of: 7.3µl of ddH_2O, 3µl of 10× buffer-1, 0.2µl of BSA 100×, 1.5µl of Kpn1 and 8µl of the PCR product. The mixture is then incubated at 37°C in a thermocycler for 16hours.

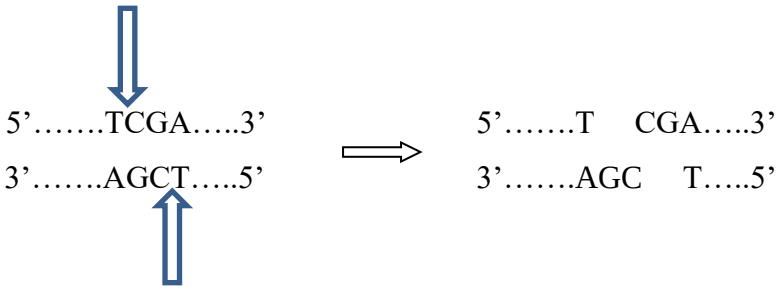
The following restriction site is recognized by the enzyme Kpn1 after digestion:



II.4.7.5.1.3. Taq1 RFLP digestion

This was done in a total volume of 20 µl which was comprised of: 7.3µl of ddH_2O, 3µl of 10× buffer-4, 0.2µl of BSA 100×, 1.5µl of Taq1 10000UI/µl and 8µl of the PCR product. The mixture is then incubated at 65°C in a thermocycler for 16hours.

The following restriction site is recognized by the enzyme Taq1 after digestion:



II.4.7.5.2. Agarose gel electrophoresis

This method was used for both the separation and analysis of DNA fragments, whose principle was based on the migration of these DNA fragments through the gel under the influence of an electric field. Migration was inversely proportional to the molecular weight of the DNA.

RFLP products were analyzed using a minigel prepared thus: 1g of agarose was weighed and and place into a 250ml beaker and 50ml of TBE added to it. The mixture was heated on a bunsen burner until a transparent solution was obtained. The solution was left to cool at 60°C, and 2µl of ethidium bromide added to it. The solution was then mixed mildly then poured into a mould containing combs and left to cool for about 45minutes. The gel was then placed into an electrophoretic cave containing sufficient quantity of the migration buffer (1× TBE). The combs are removed with care, forming wells. Using a micropipette adjusted to 10µl, the RFLP products were dissolved in 2.5µl of a loading bufferand then pipetted into the wells. Molecular weight markerswere also pipetted into the first and the last wells. The electrophoretic cave was then closed and connected to a continuous electric source at 70 V. The migration was then followed 45minutes later by the displacement of bromophenol blue

and cyanol xylene. The gel was then visualized using a UV trans illuminator and photos taken. This procedure was also used after PCR for the detection of NAT2 genes.

II.4.8. Statistical Analysis

Data was entered using Microsoft Access 2007, CS-Pro and analysed using SPSS. The distance migrated by each gene was measured against that of molecular weight marker using the Microsoft 2016 Excel Spreadsheet. The molecular weight of each gene was determined graphically from the curve of $\log_{10}$ of molecular weight marker against the distance of migration.

EfficacyVariables*:* PCR-adjusted parasitological cure rates were analysed using Fisher's exacttest, odds ratios for likelihood of cure with 95% confidence intervals, Kaplan-Meier survival analysis.

SafetyVariables*:* Prevalence of AEs: Fisher's exact test. Changes of biological safety tests & vital signs: Wilcoxon's Signed Rank test or paired t-test. Statistical significance was set at $\alpha = 0.05$ for all tests.

Allelic frequencies for the NAT2 gene were obtained using the Hardy Weinberg formula;

Fx = [2(nx/x)+nx] / 2n , where:
Fx = allelic frequency for x
nx/x = homozygotes for x
nx = heterozygotes for x
n = total number of patients
x= each SNP
The phenotypic frequencies were obtained by dividing the total number of a particular phenotype by the total number of patients. The relationship between the NAT2 gene and treatment outcomes was analyzed by the X^2 test. The Odds ratio was evaluated using a confidence interval of 95%, for the susceptibility of early treatment failure for fast acetylators and late parasitological failure for slow acetylators. A P-value less than 0.05 is considered statistically significant.

II.4.9. Methodology Phylogenetic

Genetic distances that have served for the establishment of the cladogram were calculated with the formula below:

- $D_{X/Y} = 1- N_{XY}/ (N_X +N_Y-N_{XY})$; $D_{X/Y}$is the genetic distance between X and Y,
- $N_X$is the number of electrophoresis bands of X,
- $N_Y$is the number of electrophoresis bands of Y,
- X and Y represent the differents haplotypes basison NAT2 genes, pfDHFR and pfDHPS. These distances no 4 have permitted to obtain the cladogram from the software Mégas 5.05.

II.4.10. Protein-protein interaction in the NAT2 protein network

The online software STRING v9.1 was used to identify the known and predicted protein-protein interaction in the NAT2 protein network. STRING is a comprehensive, authoritative database, which currently covers more than 9,643,763 proteins from 2031 organisms, mainly focusing on predicted and known protein interactions. These interactions include direct (physical) and indirect (functional) associations.

Construction of protein-protein interaction network

The protein-protein interactions of NAT2 protein network was built with STRING online software v9.1 under the active prediction methods (Gene Fusion, Neighborhood, Cooccurrence, Co-expression, Experiments, Databases and Text Mining), Medium Confidence (0.400) and no more than 10 interactions parameters.

CHAPTER THREE

RESULTS and DISCUSSIONS

III.1. Data collected from selected studies on irrational use and the presence of substandard/ falsified/counterfeit of antimalarial drugs in Cameroon

III.1.1. Literature Searches and outcomes

By using the combinations of the following search terms were *("Analysis" or "investigation" or "study", "Irrational use", "Substandard medicines" or "Falsified medicines" or "Counterfeit medicines", "Substandard drugs" or "Falsified drugs" or "Counterfeit druds", "Substandard antimalarial drugs" or "Falsified antimalarial drugs " or "Counterfeit antimalarial drugs ", "resistance" or "treatment failure"*), more than a million records were identified in the selected databases. After screening of titles and abstracts of these records, and according to the heterogeneity in the methodology used and in data presentation, the reseach team decided to select and comment on some interesting published studies that emphasized the irrational use of the medicines and its impact, by the possible presence of fake antimalarials.

III.1.2. Comments on selected studies from systematic review

Study by Sulaf and collaborators (2016)

This survey screened a group of people using internet and he found that 208 (65.0%) of them were really purchasing lifestyle products from the web; Amongst them, only 14.7%, 47/320 went to online pharmacies while a very small proportion (<10%) used a “drug” website. Some respondents claimed they could identify countries of origin of online pharmacies, which were mainly the United Kingdom (23.8%, 76/320) and United States (5.3%, 17/320). Others reported that sources for online pharmacies were Australia, Canada, China, Czech Republic, Finland, Germany, Greece, Holland, India, Pakistan, Philippine, Singapore, Sweden, Switzerland, and Thailand. Among the respondents who already bought medicines from UK online pharmacies, only 53 (16.6%) could recognize the Medicine and Healthcare Regulatory Agency (MHRA) logo. Moreover, only 17 (5.3%, 17/320) of respondents had consultations with a doctor at the online pharmacy. In addition, a lower percentage of respondents obtained information on lifestyle products from interacting with health care professionals (24.1%,

77/320), magazines (17.8%, 57/320), and TV (13.1%, 42/320). However, Medicinal products represented the lowest percentage of the purchased lifestyle products (11.9%, 38/320) opposite to cosmetics and other lifestyle products sold in the Internet (*Sulaf et al., 2016*).

Most respondents (62.8%, 201/320) were aware of counterfeit lifestyle products being sold via the Internet. However, the sources of information regarding product counterfeiting varied between respondents. In the majority of cases, respondents relied on information from Internet websites/drug forums (17.5%, 56/320), TV (16.9%, 54/320), and family/friends (10.9%, 35/320). Also, 25 (7.2%) respondents claimed that awareness of counterfeits is "common sense." Moreover, 13 (4.1%) respondents gained knowledge about counterfeiting from magazines and newspapers. Furthermore, 7 (2.2%) respondents knew about counterfeiting from their job within a health care setting. Education was not a major source, as 15 (4.7%) respondents claimed they learned about counterfeiting in school/university degree and only 6 (2.0%) read scientific articles on the topic (Sulaf *et al.,* 2016).

Additionally, we learnt that advice received from health care professionals was not enough regarding counterfeiting. Only 2 reported that their general practitioner and pharmacist explained product counterfeiting to them. Respondents were aware of the risks associated with the counterfeit products. However, 45 (14.1%) said that it was acceptable to take the risk of buying potential counterfeit products in case of emergencies such as medicine shortage or poor finance (Sulaf *et al.,* 2016).

Study by Onwubiko and collaborators (2014)

This study revealed that consumers do not significantly adopt protective measures against the unethical marketing of medical drugs; they found in consumers the observations below:

- Willingness to buy drugs from any source as long as their prices are cheap and affordable.
- Recognize that buying drugs only from authorized pharmacies and chemists stores would protect them from the dangers of counterfeit drugs.
- The high costs of genuine drugs in these authorized outlets created the opportunity for the consumers to consider cheaper drugs products alternatives.
- Rarely observe drug packages closely or read instructions on the inserts before consumption.

- NAFDAC numbers no longer guaranteed genuine drugs in the drug market: Some have encountered counterfeit drugs that possessed NAFDAC numbers.
- The consumers were not familiar with the use of technological devices like MAS and Tuscan to detect counterfeit drugs at the point of purchase.

However, they also discovered that:

- The non-professionals dominated the drug business as importers, wholesalers and retailers in the open drug markets. These unqualified people have little interest in terms of compliance with professional standards and their commercial interests are motivated by the desire to get-rich quickly (Ehrun *et al.*, 2001).
- More than 64% of fake and substandard antimalaria drugs are in circulation in Nigeria (Oloja *et al.*, 2011).
- Public health security requires that consumers take steps to protect themselves from counterfeit medicines. The change in consumer attitudes and behavior towards unethical drug marketing could certainly facilitate a short lifespan for this inhuman enterprise in the southeastern states of Nigeria

Study by Mbacham and collaborators (2012)

This study indicated that of the 1000 questionnaires sent out, 943 students responded with 79% (746) living in urban communities and the rest in rural settings. Most (67% (643)) of the students had a secondary level education. About 63% of the students interviewed were males. The average age of respondents was 18.6years. Even though quite a good number (89% (842)) of the respondents go to a recognised health facilities or pharmacies to seek treatment for fever, there is still some 9.7%(92) of this population who go to street vendors and traditional healers. Of the 81% that agreed to have suffered from other diseases in the past, 94% sought treatment from recognised health facilities and only 4.5% said the go to road vendors or traditional healers. However, it is surprising that, though respondents claimed they go to recognised health facilities for treatment, such a great number of them (37.2% (305) take a medication anytime they feel feverish. This suggests that most of the respondents are still visiting road vendors who are easily accessible to satisfy their feverish conditions. Again in this population that visited recognised health facilities, 45% suspected they had malaria but however they reported they took mainly antibiotics (42%) and sparingly took anti malaria (29%). There is therefore auto medication taking place in the communities with the help of non-qualified suppliers; About 88%(818) of the respondents reported they knew the

symptoms of malaria and 93% of them said they went to either a pharmacy or a health centre for treatment (Mbacham *et al.*, 2012).

In Cameroon, when a daily provision is needed, very often, the young ones of the family are sent to purchase it. Considering this, if these ones are aware of the distribution of fake medicines and are able to identify, they could save their families. In the school setting, a Cameroon Baptist Convention Health Board (CBC) team sampled various anti-infectives from kiosks in and near the town of Tiko (South West Region) and found expired packs of antimalarials (sulfadoxine-pyrimethamine) and one brand that contained no active ingredient. The same shops were also selling "completely degraded" tablets of penicillin. These stands are found around schools and serve as the primary self medication option for children who fall ill in schools. In Yaoundé and Bamenda, staff from diocesan pharmacies and faith-based healthcare centers discovered that in three leading ACT antimalarial brands, some batches contained no active pharmaceutical ingredients. Furthermore, an outdated generic SP-type of antimalarial medicine with no pyrimethamine and traces of sulfadoxine was found. Whether this was based on malpractice or outright fraud may be decided in court; the end result for patients, however, was life-taking rather than life-saving (Mbacham *et al.*, 2012).

Study by Ngono and collaborators (2018)

This study on prescription habits, evaluated the practices and mechanisms implemented to promote the rational use of drugs in health facilities located at the outskirts of Yaoundé. Prescribers were evaluated according to the usage of standard therapeutic protocols for malaria control, particularly the indicator linked to " *proportion of conform prescriptions for malaria control"*; among prescriptions in general, malaria control protocols were the main ones: 34% (204/600) of prescriptions had at least one antimalarial treatment; 80,4% (164/208) of prescriptions were filled without screening *Plasmodium falciparum* screening ; only 22 patients were screened for *Plasmodium falciparum* before their prescription (Table 5).

Table 5: Characteristic of prescriptions for malaria treatment

Characteristics	Frequence	Percentage (%)
Prescription with at least one Antimalarial	204	34 (204 /600)
Prescription without Plasmodium falciparum screening	164	80,4 (164/204)
Plasmodium falciparum screening before prescription of one Antimalarial	22	10,8 (22/204)

Percentage of prescriptions on malaria treatment conform to Malaria therapeutical protocol standards

Analysis of prescriptions for malaria cases shows that ACT represents 70% (113/164) of antimalarials used for treatments; Artemether-Lumefantrin represented 68,9% of ACTs prescriptions; only 21 patients had received Artesunate-Amodiaquine.

Table 6: Presentation of malaria treatment prescriptions by types

ANTIMALARIAL	MOLECULES	FREQUENCE	TOTAL	PERCENTAGE
MONOTHERAPY	Artemether	30	51	31,1%
	Quinin	21		
COMBINATION (ACT)	AL	72	113	68,9%
	AS-AQ	21		
	DHA-PPA	15		
	AS-MQ	5		

III.1.3. Comments on selected studies based on situational analysis of the presence and extent of Substandard/ Falsified/ Counterfeit Antimalaria drugs

With regard to the issue of the circulation of counterfeit and substandard medicines, it is clear that the presence of these products is a direct consequence of the irrational chronic behavioral use of medicines, especially for antimalarials. The above factor is strongly reinforced by the weakness of the pharmaceutical regulations; particularly access, quality assurance and the use of medicines. The use of ACT'S has been accompanied by a drastic reduction in morbidity

and mortality due to malaria in many endemic areas. The first cases of clinical failures at ACT'S have been identified in South East Asia (Pradines *et al.,* 2010). The resistance of the *P. falciparum* parasite to artemisinin was detected in Cambodia, Myanmar, the Lao People's Democratic Republic, Thailand and Viet Nam (Maillard *et al* ., 2015) in the year 2004.

The purchase of counterfeit medicines is spreading rapidly around the world with an increase trend at an alarming rate in the production, distribution and consumption (WHO, 2015). In the early 1990s, Cordell and colleagues reported that despite concerted efforts and global legal sanctions against the manufacture and consumption of counterfeit products, the problem continues to spread rapidly (Cordell *et al.,* 1996).

Global Case

However, in measuring the extent of counterfeit medicines, the most widely cited estimates ranging from 5% to 10% of the global market (Africa IRS, Nov 18, 2016 - Ingelman-Sundberg et al., 2005), rising to 25% in Least Developing Countries (LDCs) (Songue *et al.,* 2013; Barnes *et al.,* 2007; Relling, 1989; Staehli *et al.,* 2013).

By analyzing data from 320 respondents and results from the study related to *"consumer and patient knowledge, behavior and attitude toward medicinal and lifestyle products purchased from the internet":* (Sulaf *et al.,* 2016)
The respondents included 91 females (28.4%), 227 males (70.9%). The majority (62.5%, 200/320) of the respondents were in the 18-25 years' group, 47 (14.7%) in the 26-33 years' group, and 43 (13.4%) in the 34-41 years' group.

Most of the respondents were British (78.1%, 250/320), followed by Europeans (8.8%, 28/320), Asian (7.5%, 24/320), African (1.3%, 4/320), Australians (0.9%, 3/320), and Americans (0.63%, 2/320). In addition, the majority of the respondents were residents of the United Kingdom (85.0%, 272/320), Asia (6.3%, 20/320), and Europe (3.4%, 11/320).
The educational level among the respondents was mainly at a higher degree level or above; 149 (46.6%) of the respondents had at least a Bachelor's degree.

In relation to the number of languages spoken among respondents, 88.4% (283/320) were monolingual and spoke English only. The remaining respondents were bilingual or trilingual and spoke the following languages in addition to English: Arabic, Danish, Dutch, French,

German, Greek, Italian, Mandarin, Nepalese, Russian, Somali, Spanish, Swedish, Thai, Turkish, and Urdu.

These data demonstrate that internet plays a major role in the dissemination of medicinal products with many advantages over traditional market places (Songue *et al.*, 2013; White *et al.*, 2008). In this line, internet offers a quick, easy, and more convenient way for purchasing medicinal products. Even some regulated medicines without the need for a prescription (Barnes *et al., 2007,* Ingelman-Sundberg *et al.,* 2005). With internet orders can be placed from home and at any hour of the day, privacy is preserved with online purchases compared to face-to-face purchases (Relling *et al.,* 1989), saving the consumer potential embarrassment. Additionally, internet provides more detailed information about the products and reduces visits to health care professionals and community pharmacies (Ingelman-Sundberg *et al.,* 2005).

Quatari Case

Investigations on the vulnerability of the Quatari pharmaceutical market, with one hundred ninety questionnaires distributed to both community pharmacists and public (Abubakar *et al.,* 2018), demonstrate that only questions about awareness towards counterfeit medicines, its societal consequences, the effect of price affordability and the effect of pressure exerted by relatives and friends on purchase intent of counterfeit medicines gave significant difference between public and pharmacists (p-value ≤ 0.05).

These results coupled with the neighbouring environment of Qatari, showed wide scale of the phenomenon, not only in the volume but also in the area covered. According to the Pharmaceutical Security Institute, all regions of the world experienced a pharmaceutical crime incident (Marwa *et al.,* 2014). However, greater interests in understanding consumer behaviour with regard to purchasing counterfeit goods have to be developed. The apathy of drug consumers to form associations to fight unethical drug marketing and promptly report incidents of counterfeit drugs to regulatory agencies has frustrated efforts to protect consumers. Chukwuemeka and collaborators (2011) stated that the health policies, especially the Roll Back Malaria program have been negatively affected by the high incidence of counterfeit drugs.

Cameroon Case Study

About 25% of the Cameroonian population purchases medicines from informal suppliers. In North-West Cameroon, informal providers include sellers of patented medicines. Unlike neighboring Nigeria, where patented drug sellers are officially sanctioned and have a recognized role in the health system. However, little attention was paid to these actors until recently, when the debate on regulating the place of drug sellers in the Cameroonian health system resurfaced (Ongolo- Zogo and Bonono 2010). In Cameroon, a growing recognition of the importance of drug sellers has emerged alongside hypotheses that, their motivations and capacities are mainly linked to maximizing profits (Hughes *et al,* 2013).

Medicine sellers in the study by Hughes and collaaborators (2013) were varied, yet distinct group. They saw themselves as closely integrated in the social and medical landscapes of clients. Although some client interactions were described as simple sales, many respondents presented themselves as gatekeepers of medicines and knowledge, reflecting a conceptualization of the distinctness of medicines over other commodities. Acknowledgement of limits in knowledge and resources led to recognition of the need for formal healthcare providers and justified a restricted scope of practice and the need for referral. Motivation was derived from a desire for both financial and social capital combined with a proximity to medicines and repeated exposure to ill health. Medicine sellers suggest that they do not aspire to be doctors and emphasize the complementary, rather than competitive, nature of their relationship with formal providers. There are challenges and opportunities of characterizing medicine sellers as a distinctive group of ‘first aiders’ in these settings (Hughes *et al.,* 2013).

The study by AbubAkr and collaborators (2018) investigated the vulnerability of the Qatari pharmaceutical market through investigating perceptions of suppliers (pharmacists) and consumers (public) about counterfeit medicines. it was a cross-sectional study conducted using a prevalidated questionnaire to collect information about demographic characteristics, attitude, subjective norm, motivation, and behavioural intentions of pharmacists and public regarding counterfeit medicines on a Likert-scale. A total of 190 questionnaires were distributed conveniently to the public and community pharmacists. One hundred ninety questionnaires were distributed to both community pharmacists and public, however, only 167 (87.8%) were collected which resulted in response rate. Most of the results of the survey (41 items) were not significant. Only questions about awareness towards counterfeit medicines, its societal consequences, the effect of price affordability and the effect of pressure exerted by

relatives and friends on purchase intent of counterfeit medicines gave significant difference between public and pharmacists. Overall findings suggested that there is no significant difference between pharmacists and public in the way they perceive counterfeit and substandard medicines. Both of them have the same susceptibility level toward counterfeit medicines. Both pharmacists and public have low-level of knowledge and moderate level of vulnerability to counterfeit and low-quality medicines (AbubAkr *et al.,* 2018).

III.1.4. Comments on the study by Gaurvika and collaborators (2012)

After reviewing published and unpublished studies reporting chemical analyses and assessments of packaging of antimalarial drugs, of 1437 samples of drugs in five classes from seven countries in southeast Asia, 497 (35%) failed chemical analysis, 423 (46%) of 919 failed packaging analysis, and 450 (36%) of 1260 were classifi ed as falsified. In the other hand, a study on 21 surveys of drugs from six classes from 21 countries in sub-Saharan Africa, revealed that 796 (35%) of 2297 failed chemical analysis, 28 (36%) of 77 failed packaging analysis, and 79 (20%) of 389 were classified as falsified. Data were insufficient to identify the frequency of substandard (products resulting from poor manufacturing) antimalarial drugs and packaging analysis data were scarce. These results showed that poor-quality antimalarial drugs are still circulating within Africa and south East Asia; this lead to state of drug resistance and inadequate treatment to combat malaria (Gaurvika *et al.,* 2012).

Factors contributing to the production and trade of poor-quality antimalarial drugs are:

Extended self-prescription, difficult to check their quality, Trade takes place in free zones or free ports with minimum regulations, National and global drug legislation is weak or non-existent, with lax implementation and quality control and few legal sanctions, Lack of political will and cooperation of stakeholders, Proliferation of small pharmaceutical companies without adequate quality assurance, Expensive drugs with large profit margins, Poor knowledge of the consumer and health staff about the authenticity of the product and out of stock, theft and irregular supply of antimalarials.

Effects of poor-quality antimalarial drugs are:

Increased morbidity and mortality; Financial consequences for patients and their families, health-care systems, and the pharmaceutical companies producing the genuine product; Loss of confidence in pharmaceutical brand, drugs, pharmacies, and health-care providers; Drug

resistance e.g: loss of valuable drugs, false alarm of resistance, undermining of drug-resistance studies.

III.1.5. Comments on the Quality of Anti-malarials in Subsaharan Africa

Study by WHO, (2013)

This survey aimed to evaluate the quality of selected antimalarials in six countries of sub-Saharan Africa (Cameroon, Ethiopia, Ghana, Kenya, Nigeria and the United Republic of Tanzania). These countries have been supported by WHO to strengthen their regulatory controls over antimalarial products. The survey was organized independently of manufacturers of antimalarial medicines (WHO, 2013). The survey was conducted in the context of cooperation between WHO and national drug regulatory authorities in participating countries. In individual countries substantial differences in failure rates were observed. It is a positive observation that in Kenya and Tanzania the quality of antimalarials seems to be reasonably under control. Although no failing sample was detected in Ethiopia, it is of concern that a high proportion of samples of collected antimalarials were not registered (41%), and this suggests vulnerability of the market towards penetration of products with unknown properties. In other countries results are less positive because of a substantial proportion of samples that did not comply with pre-set specifications (WHO, 2013).

In Nigeria, the country with the highest incidence of failing samples according to the survey results (63.9%), the possibility to be treated with an antimalarial medicine complying with international quality standards is less than that of receiving substandard medicine. In Ghana and Cameroon (failure rates 39.5% and 36.6%, respectively), the patient has an approximately 60% chance of obtaining medicine of good quality (WHO, 2013). In these countries the strengthening of regulatory systems and market supervision seem to be of primary importance. It appears that there is a trend of higher failure rates among domestically manufactured products compared to imported ones. This indicates the need to strengthen monitoring of domestic production in countries with pharmaceutical manufacturers, and to apply the same regulatory standards for domestically produced and imported medicines. So far medicines manufactured in the countries participating in the survey appeared in general very rarely on the market in any other than the producing country. The solution to prevent the movement of substandard drugs among countries is again competent regulatory supervision of domestic manufacturers and domestically produced medicines, together with regulate

oversight over imported medicines and cooperation between regulatory bodies. Low failure rate was specifically observed for imported products manufactured by established globally acting manufacturers and for products prequalified by WHO. Although overall survey results indicate a relatively high proportion of anti-malarials that are outside specifications set up in recognized pharmacopoeias, survey outcomes should not be generalized as 'catastrophic'. In several countries results of quality testing were quite encouraging and many detected non-compliances were not extreme. Moreover, observed quality failure rates cannot be always directly related to therapeutic failures of these medicines. The information obtained through the survey has led to a better understanding of the quality profile of antimalarials in sub-Saharan Africa (WHO, 2013).

Th rise in rates of substandards drugs in Sub-Saharan Africa is presented in Table 7.

Table 7: Rise in rates of substandard drugs in Sub-Saharan Africa

Region/Country		% Off limit	Source
Angola, Burundi, DRC	CQ, Q, MQ, SP	46%	*Gaudiano et al., 2007*
Burkina Faso	CQ, Q, SP, MQ, AL	42%	*Tipke et al., 2008*
Cameroon	CQ, Q, SP	12 - 74%	*Basco, 2004*
Ghana	AS	82%	*Ofori-Kwakye et al., 2008*
Kenya	CQ, Q, SP	42%	*Chepkwony et al., 2007*
Nigeria	AS, AQ, CQ,	15-21%; 32%	*Aina et al., 2007*
GH, KN, NG, Tz, Ug, Rw	AQ, AS, MQ, DHA, AL	32-38%	*WHO, 2003*
Senegal	AQ, SP, CQ	35-55%	*Smine, Diouf& Blum, 2002*
Sudan		12-22%	*Alfadl et al. (2006)*
Tanzania	SP, AQ, Q, As	12%	*Kaur et al. (2008)*
Uganda		39-51%	*Ogwal-Okeng et al., 2003*

Legend: *Specifications were for content of active ingredients, non-declared active substance, dissolution profiles*

III.2. Quality control of ASAQ combinations in Cameroon

Quality Control of medicines could link to resistances, which are responsible for treatment failure.

Our study was a cross-sectional experimental study that took place over a period of five months covering the period from 03 January to 30 may 2017.
The work took place at LANACOME, the National Drug Quality Control and Valuation Laboratory • **Inclusion criteria**: Were included in the study the combinations of ACT in tablet form and based on Artesunate+Amodiaquine marketed by wholesale distributors, hospitals, health centers and regional funds for the promotion of health. .

• **Non-inclusion criteria**: Artesunate+Amodiaquine tablets from the illicit market and other dosage forms were excluded.

Equipment and Materiel

Laboratory reagents:

¬ Acetonitrile R for HPLC

¬ Phosphatemonopotassium anhydrous

¬ SoduimR octanesulfonate

¬ Methanol

¬ Ammonia

¬ Water R

¬ Phosphoric acid

¬ Amodiaquinehydrochloride Reference chemical substance with a certificate of analysis (batch number: 1921160), Ref Stockholn, Sweden, SN: 2371035 EDQM.

¬ Artesunate chemical reference substance with a certificate of analysis. (batch number: 45422), CS30025-F67081stasburg (France) EDQM.

-Laboratory equipment:

¬ Test tubes

¬ Pestle and Mortar

¬ Volumetric pipettes 2ml, 3ml

¬ Volumetric flask 20ml, 25ml, 50ml, 100ml

¬ Graduated cylinders

¬ Micropipette

¬ Plate: Silica gel

¬ Electric balance: METLER TOLEDO X104 brand SANTRUIS with an accuracy of 1 / 10th of a milligram

¬ UV lamp (254nm, 366nm)

¬ Spatulas

¬ Vortex

¬ UV-visible spectrophotometer, type: Lambda 25, Serial No 501S10072802, Manufacturer: Perkin Elmer.LAN 064

¬ HPLC with the Agilent Technologies brand iodine array for detector model: Infinity 1260, DEAEQ05576 series

¬ Disagregation Apparatus: ERWEKA brand, ZT3 model,

¬ Dissolutest: Sotax brand

¬ Friabilimeter: Sotax brand, FT2 model

¬ Ultrasonic baths

¬ Filter paper

¬ 10 ml syringes

Method:

First of all we proceeded to the sampling of our various drugs then, the dosage control protocol was that developed and validated in the laboratories of the ANSM formerly called AFSSAPS which allowed the simultaneous determination of the two principles active by the same method (High Performance Liquid Chromatography). But before arriving at that we carried out physical tests (i.e. the control of the labeling), the pharmacotechnical tests; after we made an identification of the two active principles by Thin Layer Chromatography (TLC), as well as the Dissolution test of the amodiaquine, and in fine the Physico chemical tests.

III.2.1. Distribution of samples

According to the distribution of samples by continent of origin, 67% of the samples came from Asia; followed by North Africa with 27% and finally Europe with 7%. By country of origin, most of the medicines came from India (60%). By dosage, the adult dosage (100/270 mg) occupied the largest part (60%). According to the sampling circuit, the public circuit occupied the largest part with 67% of the samples and finally, according to the site of purchase, the hospitals were the most represented (table 8).

Table 8: Distribution of Samples

According to the continent of origin								
Asia		**Africa (Northen)**		**Europe**				
10 (66.66%)		04 (26.67%)		01 (6.67%)				
According to the country of origin								
India		**China**		**Morocco**		**Germany**		
09 (60%)		01 (6.67%)		04 (26.66%)		01 (6.67%)		
According to the dosage								
Infant 25/67.5		**Child 50/135**		**Teenager 100/270**		**Adult 100/270**		**Adult 100/300**
02 (13.33%)		02 (13.33%)		04 (26.67%)		06 (40%)		01 (6.67%)
According to the sampling circuit								
Private Circuit				**Public Circuit**				
05 (33.33%)				10 (66.67%)				
According to the purchase site								
Hosp1	**Hosp2**	**Hosp3**	**Frps1**	**Frps2**	**Heath C**	**Pharm**	**Wholesaler**	**Wholesaler**
03 (20%)	01 (6.66%)	01 (6.66%)	02 (13.34%)	01 (6.66%)	02 (13.34%)	01 (6.66%)	02 (13.34%)	02 (13.34%)

Hosp: hospital; C: centre; Pharm: pharmacy

II.2.2. Physical analysis

Sample Label Analysis: Primary Packaging

The analysis of the primary packaging, revealed that all samples had a record in English and French with aluminum or PVC packaging. It should be noted moreover, that all the collected samples did not have VISA in Cameroon. These are samples 1, 2, 3, 5, 6, 7, 8, 9, 10. These batches came mainly from INDIA and were all collected from hospitals (table 9).

Table 9: Sample Label Analysis: External Packaging

Sample Number	DCI	LN	MN	ED	DM	Dosage	Circuit	Conclusion
1	+	+	+	+	+	+	Public	Conforms
2	+	+	+	+	+	+	Public	Conforms
3	+	+	+	+	+	+	Public	Conforms
4	+	+	+	+	+	+	Public	Conforms
5	+	+	+	+	+	+	Public	Conforms
6	+	+	+	+	+	+	Public	Conforms
7	+	+	+	+	+	+	Public	Conforms
8	+	+	+	+	+	+	Public	Conforms
9	+	+	+	+	+	+	Public	Conforms
10	+	+	+	+	+	+	Public	Conforms
11	+	+	+	+	+	+	Privé	Conforms
12	+	+	+	+	+	+	Private	Conforms
13	+	+	+	+	+	+	Private	Conforms
14	+	+	+	+	+	+	Private	Conforms
15	+	+	+	+	+	+	Private	Conforms

ED: expiration date; MN: manufacturer's name; LN: lot number; DM: date of manufacture; +: present

III.2.2.1. Organoleptic characteristics

It should be noted that samples number 8, 9 and 10 adhered in the blisters. This suggests poor conservation or even problems related to manufacturing. In addition, the samples numbers 4, 10, 13 and 15 crumbled in the blisters, revealing a failure in the formulation and technology.

III.2.3. Pharmacotechnical Analysis

III.2.3.1 Average mass uniformity

Since the average mass is 939.06 mg, which is greater than 250 mg, the limit difference is <> 5% defined in the European Pharmacopoeia, 9th edition, Volume 1.

III.2.3.2. Mass uniformity

"**Simple tolerable deviation**": ± 5 %

E% = $\frac{939.06\ x\ 5}{100}$= 46,953

Upper Limit = 939.06 + 46.953 = 986,013mg

Low Limit = 939.06 – 46.06 = 892.107mg

892.107mg <939.06mg <986,013mg

Number of tablets out of limit: 00

The mass data for the fifteen batches are as follows: 1 (939.06 mg); 2 (942.238 mg); 3 (943.09 mg); 4 (176.43 mg); 5 (486.88 mg); 6 (938.06 mg); 7 (938.43 mg); 8 (942.71 mg); 9 (943.41 mg); 10 (235.89 mg); 11 (727.56 mg); 12 (292.21 mg and 508.91 mg); 13 (707.21 mg); 14 (702.47 mg) and 15 (353.64 mg).

The tablets tested did not reveal any significant variation in average weights of all the samples having passed this test. The results suggested that the rheological characteristics of the powders or granules should be satisfactory, leading in all cases to uniformity of the masses according to the specifications of all the samples, as presented on figure 10 below.

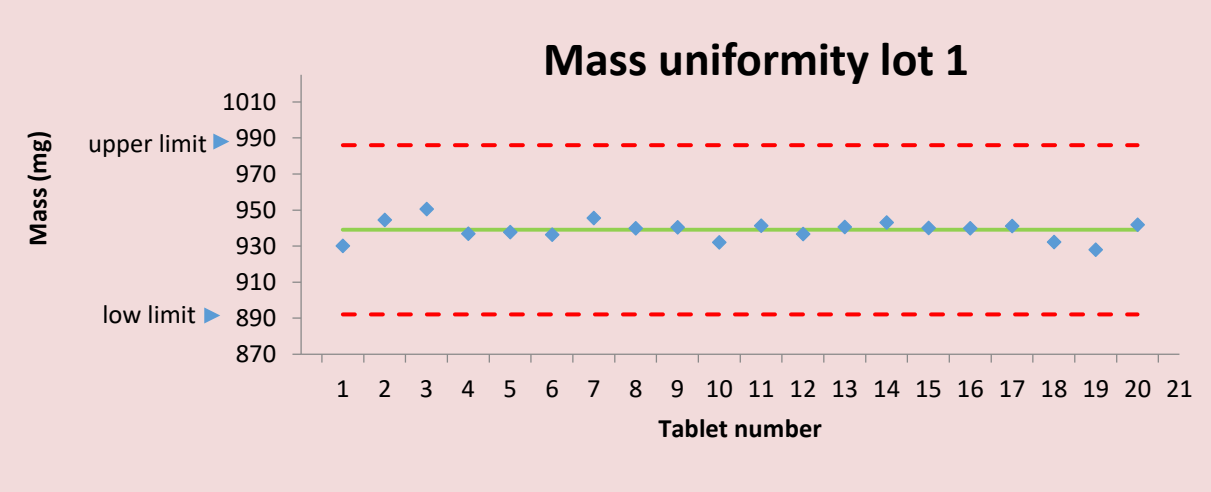


Figure 10 : Presentation of mass uniformity

III.2.3.3. Disintegration Test

For the disintegration tests, all the samples were compliant with a disintegration time of less than 6 minutes, as it is showed on table 10 below.

NORM ≤ 15 minutes

Table 10: Results of the Disintegration Test

Batch Nº	Disintegration Time (min)	Conclusion
1	3	Conforms
2	3	Conforms
3	4	Conforms
4	<1	Conforms
5	3	Conforms
6	3	Conforms
7	3	Conforms
8	5	Conforms
9	5	Conforms
10	3	Conforms
11	<1	Conforms
12	<1	Conforms
	<1	Conforms
13	1	Conforms
14	1	Conforms
15	1	Conforms

III.2.3.4. Friability test

Of the fifteen (15) batches analized, only two (02) were not conform.

Two batches out of the 15 analyzed did not pass the test. These are batches 4 and 10 which are infant's presentation, giving a percentage of 13% of non-compliance to the friability test. These batches came from hospitals and health centers. This situation poses a quality risk for child dosing. The figure is presented on table 11 below.

Table 11: Friability test results

Batches Number	%Loss	Conclusion
1	0.22	Conforms
2	0.29	Conforms
3	0.25	Conforms
4	1.03	non-compliant
5	0.20	Conforms
6	0.13	Conforms
7	0.13	Conforms
8	0.47	Conforms
9	0.2	Conforms
10	3	non-compliant
11	0.3	Conforms
12	0.12	Conforms
	0.14	Conforms
13	0.35	Conforms
14	0.17	Conforms
15	0.66	Conforms

Standard: ≤1%

III.2.4. Physicochemical Analysis

III.2.4.1 Dissolution test results for amodiaquine base

Although having passed the steps E1 and E2 of the dissolution test, 1 batch out of the 15 analyzed did not pass the dissolution test. This is the batch number 11. This batch from the European circuit of origin have given a normal disintegration time and its AP content was conformed. This shows the importance of the dissolution test in the Quality Control of drugs.

DILUTION CARRIED OUT: sample lot 2, dosage: 100/270 mg. The results are showed in table 12.

Table 12: Results dissolution Amodiaquine base

ECH 2	OD (ABS)	Percentage dissolution (%)	Volume of dissolution (ml)	Sample Dosage (mg)	Dilution (ml)
Std 1	0.51983		X	92.2 %	30/50 then 2/100
Std 2	0.52126				
Sample 1	0.6006	81.11	900	270	2 /50
Sample 2	0.60216	81.32			
Sample 3	0.61029	82.42			
Sample 4	0.61739	83.38			
Sample 5	0.63012	85.10			
Sample 6	0.60493	81.70			

This dilution was carried out for all 100/270 mg assays and, for all other assays the same logic was used.

Calculation of the similarity factor

Similarity Factor = (Abs Std 1) / (Abs Std 2) X (PE Std 2) / (PE Std 1)

Abs STD1 = standard absorbance 1; Abs Std 2 = standard absorbance 2; PE Std 1 = standard test sample 1; PE Std 2 = standard test sample 2

Application: similarity factor = 0.51983 / 0.52126X 30.1 / 30.1 = 0.99 which is effectively between 0.98 and 1.02: So our samples standards were prepared under the same conditions;

Formula of calculation which follows from the law of Beer Lambert:

$$E = \frac{Abs\ Ech}{Abs\ Te}\ X\ \frac{Pe\ Te}{Vd\ Te}\ X\ \frac{VEpd\ Te}{Vpd\ Te}\ X\ \frac{VdEch}{PE\ Ech}\ X\ \frac{VpdEch}{VEpd\ Ech}\ X\ \frac{(purity - water\ content)}{100}\ X\ \frac{355.9}{464.8}$$

Abs Ech = absorbance sample; *Abs Te = absorbance control;*
Pe Te = standard test sample *Vd Te = sample control dilution volume;*
VE pd Te = sample test first dilution of control; *Vpd Te = volume first dilution of control;*
Ech = Test sample;

*355.9 / 464.8 Represents the **conversion factor** of hydrated Amodiaquine dihydrochloride to Amodiaquine based (355.9 = molecular weight of Amodiaquine base, 464.8 = molecular weight of hydrated Amodiaquine dihydrochloride).*

III.2.4.2. Results identification of artesunate and Amodiaquine by TLC

The identification of the two active compounds was positive in the 15 batches analyzed by the Minilab antimalarial kit method. The figure below on which the distances traveled by the solvent, by the test sample and by the amodiaquine reference standard are visible allowed the identification of Amodiaquine (figure 11) or artesunate (in the same way) by using the calculation of the frontal ratio (**FR = d / D**) for the standard and for the test sample.

Preparation of a standard solution and the sample

After spraying our tablets in a mortar, we weighed the required quantities associated with each dosage so that at the end of the analysis we could have a concentration of 0.625mg. This weighed mass was dissolved in 10 ml of water. With the help of a pipette we took 1ml of this solution and we added 10ml of methanol.

¬ **Development**

Using a pipette, we introduced 5 ml of ethyl acetate, 20 ml of methanol and 0.5 ml of concentrated ammonia solution into the chromatography tank. Then we closed the tank to saturate the medium for 15 minutes. During this time we put spots of our different samples and reference standard on the chromatographic plate. We then dried it with a dryer and introduced this plate into the tank. We allowed to migrate for 20 minutes and we removed the plate from the tank and marked the solvent front. We let it dry at room temperature.

Detection

Our chromatographic plate was revealed with a UV lamp at 254 nm.

a) Artesunate

¬ PRINCIPLE

We extracted the artesunate from the combination with methanol.

¬ PREPARATION OF THE STANDARD SOLUTION AND THE SAMPLE

After spraying our tablets in a mortar we weighed the required quantities associated with each dosage. This weighed mass was dissolved in 20 ml of methanol.

¬ DEVELOPMENT OF THE CHROMATOGRAM

Using a pipette we introduced 18 ml of ethyl acetate, 4 ml of acetone and exactly 0.1 ml of anhydrous acetic acid into the chromatographic tank. Then we closed the tank to ensure saturation of the medium for 15 minutes. During this time we put spots of our different samples and reference standard on the chromatographic plate. We then dried it using a dryer and introduced this plate into the tank. We allowed to migrate for 10 minutes and we removed the plate from the tank and marked the solvent front. We let it dry at room temperature.

REVELATION OF SPOTS

The artesunate was developed with sulfuric acid and methanol. To do this, we filled a 250 ml beaker for this purpose, with 190 ml of methanol and 10 ml of concentrated sulfuric acid solution. We stirred and let the mixture cool. Then we sprinkled the mixture on the baking sheet and let out the excess liquid. We let the plate dry on a hot plate and as it warmed up, the artesunate stains gradually appeared in the daylight. This is presented on figure 11 below.

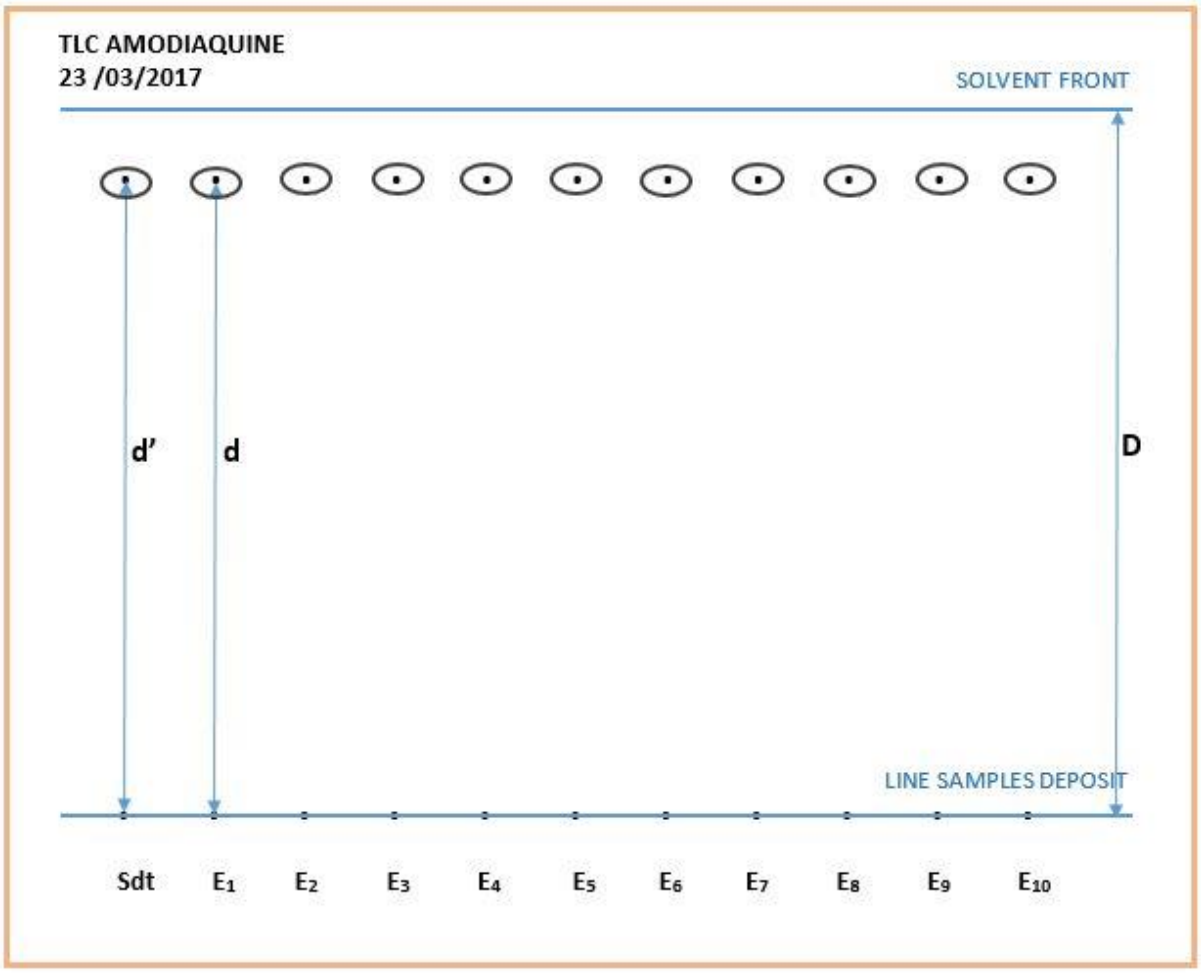


Figure 11: TLC identification plate for Amodiaquine

d = distance traveled by the sample; d'= distance traveled by the standard; D = distance traveled by the migration solvent. d: Sample1 = 12.3cm; d': std = 12.2 cm; D: solvent = 13.5 cm. FR std = 12.2 / 13.5 = ***0.90*** *and FR Sample = 12.3 / 13.5 = 0.91; with α=5%.*

Conclusion: CONFORM.

These results were confirmed by HPLC identification with retention times that were almost comparable (±5% difference).

III.2.4.3 Results HPLC assay of artesunate and amodiaquine

III.2.4.3.1 Amodiaquine

The repeatability of the standard is validated (compliant) if the Coefficient of variation (CV) is ≤ 1% according to European Pharmacopoeia, 10th Edition, Volume 1

The Value Area of the standard 2 = 12142.3525; the different measurements led us to the figure on table 13.

Table 13: Repeatability of the standard

	Repeatability of std 1
1	12181,0244
2	12186,3584
3	12171,6865
4	12160,3476
5	12146,4189
6	12154,3066
7	12194,123
Mean	12170,60934
Standard- Deviation	17,63906116
CV%≤ 1 %	0,144931619

Calculation formula of Similary Factor (SF): **Similarity Factor**

$$= \frac{\boldsymbol{Area\ STD\ 1}}{\boldsymbol{Area\ STD\ 2}} \boldsymbol{X} \frac{\boldsymbol{PE\ STD\ 2}}{\boldsymbol{PE\ STD\ 1}}$$

With: Area STD1 = Area of standard 1; Area STD 2 = Area of standard 2; PE STD 1 = standard test sample 1; PE STD 2 = standard test sample 2.

Application: **similarity factor** $= \frac{12170.60934}{12142.3525} \boldsymbol{X} \frac{88.2}{88.6} = \boldsymbol{0,99}$

Which is actually between **0.98 and 1.02** so our standards have been well prepared and under the same conditions.

Formula for calculating the content

$$E=\frac{AreaECH}{AreaTe}\ X\frac{PETe}{VdTe}X\frac{VdECH}{PEECH}X\ \frac{(purity-water\ content)}{100}\ X\ mean\ weigth\ X\ 0.766$$

Area ECH = sample area; Area Te = area of the Control; PE Te = taking test of the control; Vd Te = volume dilution of the control; Vd ECH = volume dilution sample; PE ECH = take test sample.

With the **conversion factor** of hydrated Amodiaquine dihydrochloride to Amodiaquine base is 355.9 / 464.8 = 0.766 with 355.9 = molecular weight of Amodiaquine base; 464.8 = molecular mass of Amodiaquine hydrochloride.

% CONTENT = (Emean x 100) / (batch dosage)

When Emean is the average of the three assay value of batch study

Calculation of the coefficient of variation.

$$CV = \frac{SX100}{Emean}\ with\ s = \frac{\sqrt{(x-xi)2}}{n-1} = \text{standard deviation}$$

When calculating the **Coefficient of Variation**, the value found must be CV≤ 2.41 for n = 3 These calculations are found in the table below and an example was taken to illustrate, and all the others were calculated in the same way.

TEST	AREA STD	AREA SAMPLE BATCH 6 AMODIAQUINE	PM (MG)	PE (MG)	CONTAIN		PERCENTAGE
1	12156,4809	12166,8769	938,06	234,1	248,757941		
2	12156,4809	12320,1377	938,06	234,3	251,676419		
3	12156,4809	12197,4521	938,06	234,6	248,851559		
		Mean				249,761973	92,5044345
		Standard Deviation				1,65861955	
		CV≤ 2,41				**0,6640801**	

PM = average weight; PE = test, E1, E2, E3 = tests 1, 2, 3 of lot 6

Table 14: Amodiaquine assay results by HPLC

RESULTS DOSAGE AMODIAQUINE BY HPLC							
Sample Number	**Dosage**	**contain Amodiaquine base**	**percentage found %**	**Norm of ANSM and Int. Ph**	**Std deviation**	**CV%**	**conclusion**
Sample 1	100/270	245,228	90,83	90 - 110 %	0,7448	0,303	Conforms
Sample 2	100/270	249,142	92,28	90 - 110 %	0,344	0,13	Conforms
Sample 3	100/270	240,615	89,12	90 - 110 %	0,33096	0,1375	Non-compliant
Sample 4	25/67,5	59,142	87,618	90 - 110 %	0,2428	0,4106	Non-compliant
Sample 5	50/135	122,878	91,02	90 - 110 %	0,4	0,325	Conforms
Sample 6	100/270	249,761	92,51	90 - 110 %	1,65	0,66	Conforms
Sample 7	100/270	244,229	90,45	90 - 110 %	1,15	0,4725	Conforms
Sample 8	100/270	248,789	92,14	90 - 110 %	1,33	0,5364	Conforms
Sample 9	100/270	250,695	92,85	90 - 110 %	0,8725	0,342	Conforms
Sample 10	25/67,5	60,086	89,01	90 - 110 %	0,5969	0,99	Non-compliant
Sample 11	100/270	244,25	90,46	90 - 110 %	1,065	0,43614	Conforms
Sample 12	100/300	272,787	90,92	90 - 110 %	0,3029	0,111	Conforms
Sample 13	100/270	247,825	91,78	90 - 110 %	0,79028	0,31	Conforms
Sample 14	100/270	244,896	90,702	90 - 110 %	2,04	0,83	Conforms
Sample 15	50/135	123,02	91,12	90 - 110 %	0,8425	0,684	Conforms

The 15 batches collected for the study allowed us to test 45 samples of ASAQ combinations, according to European Pharmacopoea specifications: origin 66%(ASIA); 26,6% (North Africa) and 6,6%(Europe).

The dose-dependent performance shows that in the 67% of the 100/270 mg (adult) samples collected, 20% were non-artesunate and 7% non-Amodiaquine compliant, for a total of 27% of nonconformities in the collected samples.

For the 100/300 (adult) dosage form, all the 7% collected were artesunate and Amodiaquine compliant.

For the 25 / 67.5 (infants) dosage, in the 13% collected, 13% of nonconformities were found to be Amodiaquine and all were artesunate compliant.

For the 50/135 (children) dosage, all the 13% samples collected were artesunate and Amodiaquine compliant.

In the public sector structures, in *the 67% collected, 20% of samples were respectively non-compliant with artesunate and Amodiaquine, giving a total of 40% non-compliance of the samples collected.*

III.2.4.3.2. Artesunate

As describe previously for Amodiaquine, the same methodology was use in the case of Artesunate.

Table 15: Results dosage of Artesunate by HPLC

DOSAGE ARTESUNATE BY HPLC							
Sample Number	Dosage	Contain artésunate	percentage %	Standard of ANSM and Int Ph	Std Dev	CV%	Conclusion
Sample 1	100/270	90.695	90,7	90 - 110 %	0,2826	0,311	Conforms
Sample 2	100/270	90,3626	90,363	90 - 110 %	0,038	0,04	Conforms
Sample 3	100/270	92,1672	92,17	90 - 110 %	0,8262	0,8964	Conforms
Sample 4	25/67,5	23,4821	93,92	90 - 110 %	0,0709	0,30211	Conforms
Sample 5	50/135	45,2578	90,52	90 - 110 %	0,3414	0,7544	Conforms
Sample 6	100/270	87,4726	87,47	90 - 110 %	0,555	0,6351	Non-compliant
Sample 7	100/270	90,489	90,49	90 - 110 %	0,6592	0,7284	Conforms
Sample 8	100/270	87,504	87,51	90 - 110 %	0,3625	0,4148	Non-compliant
Sample 9	100/270	88,5433	88,54	90 - 110 %	0,6899	0,779	Non-compliant
Sample 10	25/67,5	22,958	91,84	90 - 110 %	0,459	2	Non-compliant
Sample 11	100/270	90,5514	90,85	90 - 110 %	1,61	1,77	Conforms
Sample 12	100/300	90,331	90,33	90 - 110 %	1,881	2	Conforms
Sample 13	100/270	91,021	91,02	90 - 110 %	0,511	0,5611	Conforms
Sample 14	100/270	92,609	92,61	90 - 110 %	0,554	0,5988	Conforms
Sample 15	50/135	45,9522	91,91	90 - 110 %	0,6169	1,342	Conforms

The dissolution rate for Artesunate in ASAQ combination presented 26, 6% of non-conformity and 73,4% of conformity.

III.2.4.4. Conformity of the samples

Concerning the conformity of the samples according to the country of origin Germany, the batch (7% of samples)collected, were all in accordance with artesunate and amodiaquine ; China, the batch (7% of samples)collected, were artesunate and Amodiaquine compliant; Morocco, above the 4 batches (27% of samples) collected, 7% were not in conformity with amodiaquine and the rest were in conformity with artesunate; In India, in the 9 batches (60% of samples) collected, 20% were non-compliant with artesunate and 13% of samples were non-compliant with Amodiaquine , a total of 33% of non-conformities.

Conformity according to dosage shows that in the 10 (67% of samples) of tested batches (30 samples), 100/270 mg (adult presentations) were collected, (20% of samples) of 9 batches were non-artesunate and 1batch (7% of samples) non-amodiaquine compliant. A total of 27% of nonconformities of samples were collected.

For the 25 / 67.5 mg (infant's presentations), 2 batches (6 samples) were collected, 2 of them were non-Amodiaquine compliant and all were artesunate compliant.

At the end of the analyzes we found that all the nonconformities were found in the public circuit; above the 10 batches, (30 samples) from this circuit, 6 proved to be non-compliant and were also sub-standards, 3 non-compliant in artesunate and the rest 3 were non-Amodiaquine samples.

We found that all these substandard samples were imported largely from India. Above the 15 batches analyzed, 3 were non-compliant to artesunate and 2 were amodiaquine non-compliant. In all the 33% of non-conformities, we found 5 non-compliant from India; amongst the 4 batches from Morocco, 1 was non-compliant in amodiaquine, giving a percentage of 7% of non-compliance products from Morocco.

Non conformities were found mostly in ASIA. Over 10 batches (30 samples) from ASIA, 3 were non-artesunate and 2 non-amodiaquine compliant, giving a percentage of 33% of the samples collected. **North Africa with 1 Amodiaquine non-compliant sample in the 4 collected follows with a percentage of 7% of nonconformities.**

III.3. Pharmaco economics interpretation results

By analyzing database from WHO studies, it is clear that, overall, counterfeit medicines constitute a huge market estimated at more than 35 billion dollars and represent more than 15% of the world pharmaceutical market. This proportion reaching more than 60% in developing countries (Brasseur *et al.,* 1988). The WHO estimates that 50% of medicines available via internet are fake. Counterfeit antimalarial, mainly artemisinin, is a crucial in developing countries, particularly in Africa. Promoters of substandard drugs infiltrate the distribution channel with counterfeit drugs. Consumers are therefore exposed to high risks to health and safety. Public health problems such as liver damage, kidney and heart failure, disability, injury and even death have been closely associated with the use of counterfeit drugs (Akunyili, 2010). Up to 2,000 children die every day in Africa as a result of taking counterfeit malaria drugs (Rago, 2009).

The health situation became more complex in 2014 with an uncontrolled circulation of drugs in these areas of Southeast Asia and Cambodia with more than 300 drug importcompanies, 60% of which are Indian (Bourdier *et al.,* 2015). At least 40% of the specialties that were supposed to be artesunate-based contained no trace of this active substance and therefore did not provide any therapeutic benefit (Nayyar *et al.,* 2012). Cameroon, having practically no production unit, is forced to import the vast majority of antimalarials from various sources. The current practice of self-medication and the quality of antimalarial drugs from informal distribution channels in Cameroon leads to the acquisition of poor quality drugs and does not effectively fight against the disease but on the contrary, it favors an increase in the number of parasites resistant to treatment (Robert *et al.,* 2004). A study conducted in Southeast Asia in 2008 in all sales or delivery channels brought to light fake artesunate tablets. In fact, half of the samples collected were counterfeits. Some of these illicit products contained small amounts of artesunate, others showed either active compounds other than artesunate (chloramphenicol, chloroquine, erythromycin, metronidazole ...), or pharmacologically inert substances such as limestone powder. These falsifications would probably contribute to the observed resistance to treatment.

The data from the study by Mori and collaborators revealed that it was estimated at US$16.2 million, among that, the value of substandard medicines was US$13.7 million (84.4%), and for the falsified medicines US$0.1 million (1%), cosmetics with prohibited ingredients value

was US$1.3 million (8%) and other/operational costs US$1.1 million (6.6%) (Mori *et al.*, 2018). Some of the identified substandard and falsified human medicines identified were commonly used antibiotics such as phenoxymethylpenicillin, amoxicillin, cloxacillin and co-trimoxazole; antimalarials such quinine, sulfadoxine– pyrimethamine, sulfamethoxypyrazine–pyrimethamine and artemether–lumefantrine; antiretroviral drugs; antipyretics and vitamins among others. The conclusion of the study was that the economic cost of substandard and falsified human medicines and cosmetics containing prohibited ingredients represent a relatively large loss of scarce resources for a poor country like Tanzania (Mori *et al.*, 2018).

The data from the study by Nafiu and collaborators emphasized the fact that the global market volume of SFM could be up to US$200 billion, and up to 70% of the total medicines in circulation could be SFM in some parts of Africa. This dominance in the region is a clear sign of SFM proliferation, which continues to cause avoidable health hazards leading to severe adverse effects and devastating loss of human lives, by compromising treatment of chronic, infectious, and life-threatening diseases, such as malaria, cancer, pneumonia, tuberculosis, and diabetes. Besides these consequences for public health and safety, the economic and societal harms are equally serious. Although the recent advancement in detection technology coupled with increased collaborative efforts between some African drug regulatory agencies has led to a considerable success in countering the SFM pandemic (Nafiu *et al.*, 2017).

III.4. Randomized comparative controlled trial study in the Northern regions

III.4.1. Profile of the Study Population

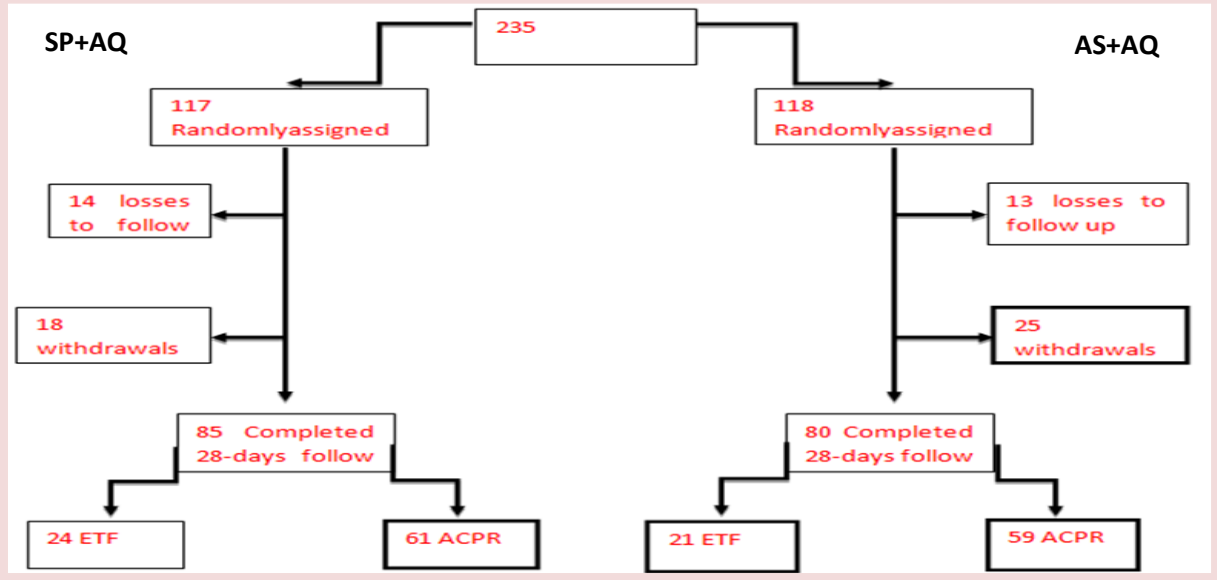


Figure 12: Consort Chart of ASAQ and SPAQ treatment groups

A total of 235 children were enrolled from both regions. The number of children randomised to the ASAQ group was 117 while those assigned to the SPAQ group was 118. There were 14 withdrawals/losses to follow up in the ASAQ group as opposed to 13 in the SPAQ group. Finally, 85 children completed the study in the ASAQ group and 80 in the SPAQ group. Follow-up was in accordance with the protocol.

III.4.2. Baseline Characteristics of the Study Population

The baseline characteristics of the population as seen in the table below shows the different characteristics of the study participants on day 0. The female to male ratio was 55:54 in the SP+AQ group and 51:58 in the AS+AQ group. Unfortunately, there were 8 unrecorded sex status in the SPAQ group and 9 in the ASAQ group. There was no significant difference in sex and age between both groups. The mean hemoglobin level at baseline was 10.24±2.3 g/dl in the SP+AQ group and 10.14±2.4 g/dl in the AS+AQ group. There was however no significant difference between the two groups (p=0.75). The mean temperature in the AS+AQ group was 38.6±0.9˚C and 37.9±0.8˚C in the SP+AQ group. This difference was also not significant between the two groups (p=0.47). This difference was not statistically significant. No significant difference was observed in the mean geometric levels of parasite counts in both

treatment groups (p=0.38). In total, **all the variables were not statistically significant in both treatment groups.** Therefore, the two groups were statistically comparable at baseline.

Table 16: Baseline Characteristics of the 235 enrolled study participants

Variable	Treatment Group		p-value
	AS+AQ	SP+AQ	
Female: Male ratio*	55:54	51:58	0.84
North: Far North	54:63	55:63	0.94
Mean age(months)±SD	48.01±28.7	46.37±31.4	0.69
Mean Hb(g/dl)±SD	10.24±2.3	10.14±2.4	0.75
Meantemperature (˚C)±SD	38.6±0.9	37.9±0.8	0.47
Weight(kg)±SD	15.31±7.5	14.92±6.1	0.68
Geometric mean Parasite count(/µl)±SD	3981±3	3801±3.1	0.38

F:M = Female: Male ratio; * indicates that sex status missing for 8 in SPAQ group and 9 in ASAQ group; Hb=Hemoglobin; SD=Standard deviation: Confidence interval = 95%; p = 0.05

III.4.3. Parasite and Fever Persistence Profiles

III.4.3.1. Persistence in Parasitaemia during 28 day follow up

Generally, by day 2, irrespective of the drug (AS+AQ or SP+AQ), at least 60% and 50% of all children in the North and Far North respectively had a complete parasite clearance. This drop was at its lowest on day 14 for both drug groups. This was followed by a sharp increase in parasitaemia in both treatment groups (Figure 13).

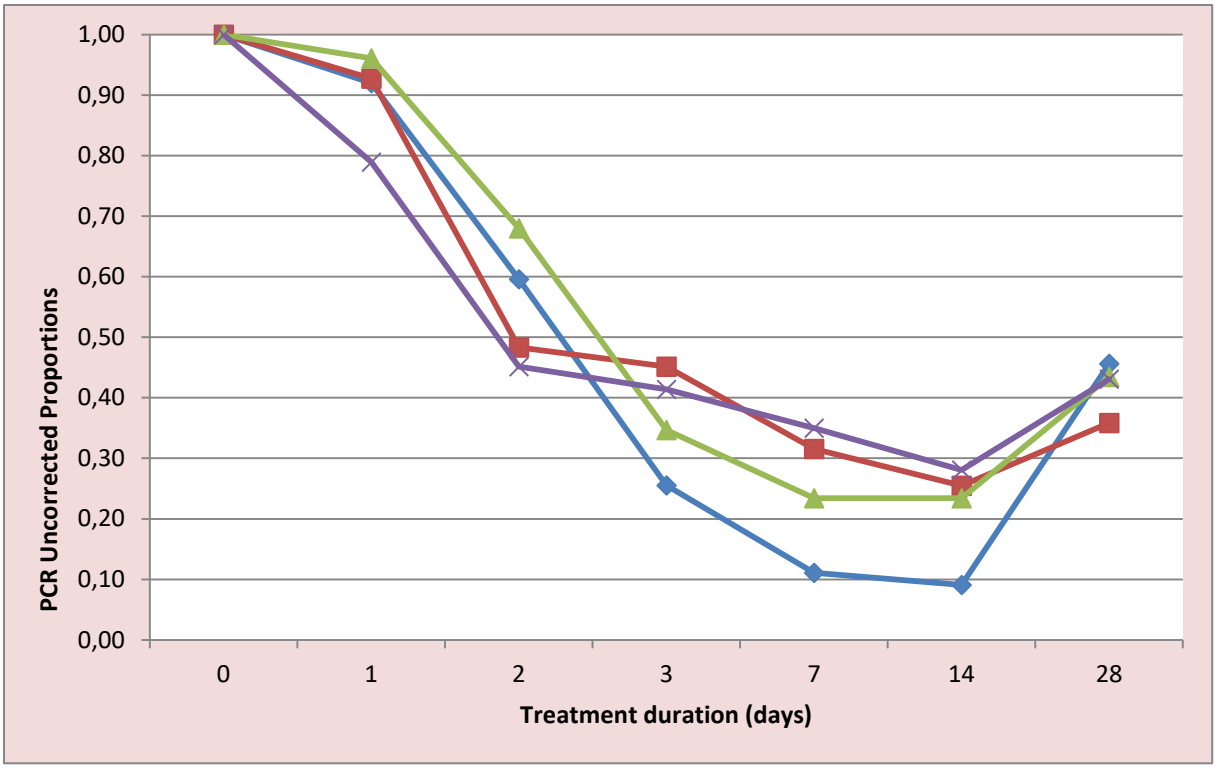


Figure 13: Proportion of children with persistent parasitaemia in the AS+AQ or SP+AQ treatment groups

Legend:

III.4.3.2. Geometric Mean Parasite Density Levels during 28 Day Follow Up Period

The geometric mean parasite density (GMPD) for the 235 children recruited on Day 0 was 4169/µl and by Day 3, the GMPD had fallen to 589/µl, therefore giving a percentage success parasite clearance rate of 86%. The parasite clearance success rates for the other days were as follows: Day 1(50%), Day 2(80%), Day 3(86%), Day 7(90%), Day 14(90%) and Day28 (40%).

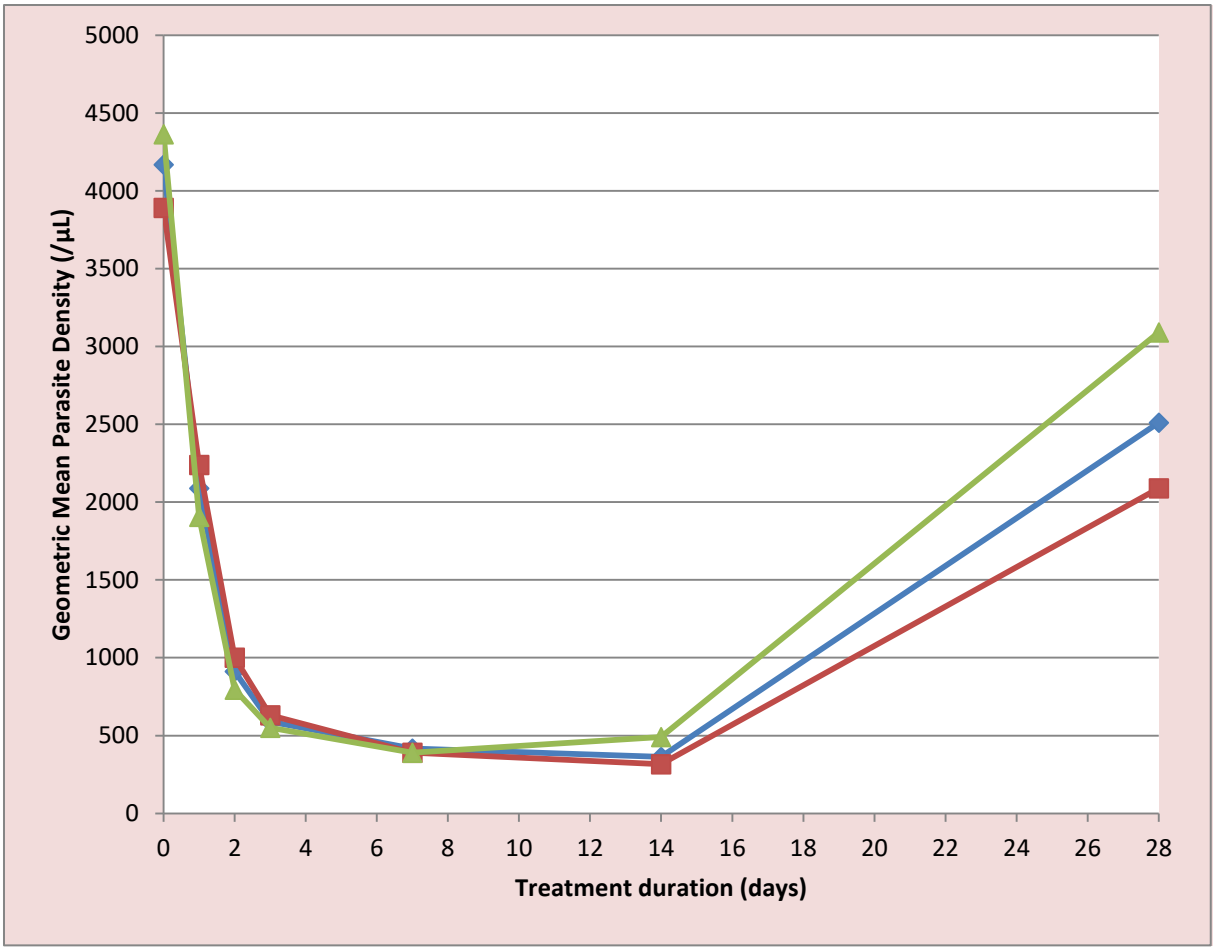
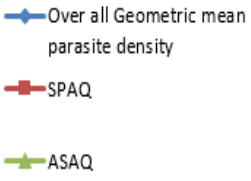


Figure 14: Geometric mean parasite density in the ASAQ or SPAQ treatment groups

Legend:



We observed from Day0 to Day3 a rapid decrease of the Geometric Mean Parasite Density level; then it went down slowly uptil Day 14: following a sharp increased uptil Day28. This is presented on figure 14 above.

III.4.3.3. Evolution of Clinical Fever

The clinical fever relief pattern was not different across the two treatment groups at baseline and by day 2, a very small proportion of children had clinical fever in both groups. However, on day 14, there was an increase in the proportion of children with fever in the ASAQ group, followed by a sharp decrease on day 28. While the SPAQ group observed a constant decrease in the proportion of children with clinical fever (figure 15).

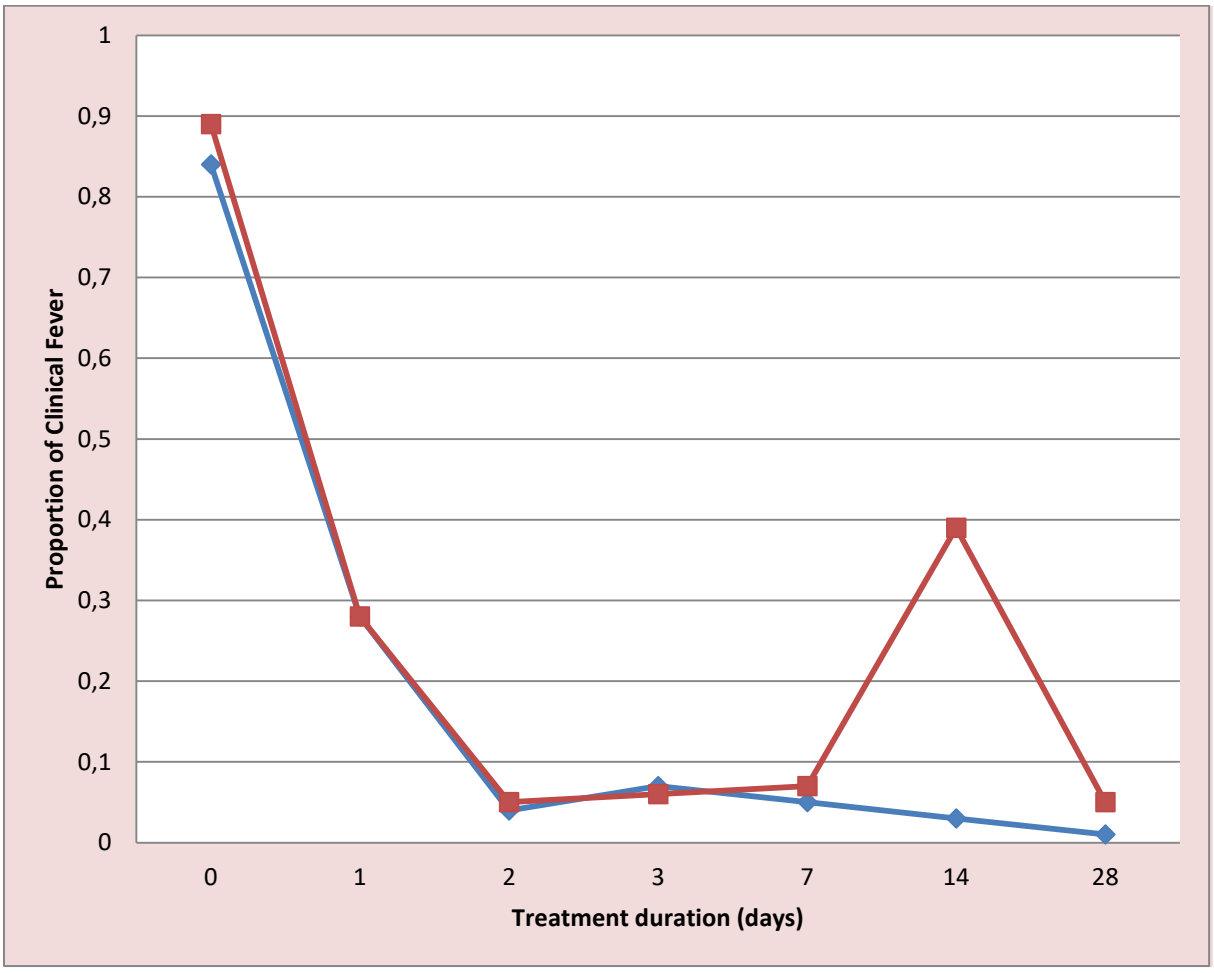


Figure 15: Proportion of Clinical Fever in the ASAQ or SPAQ treatment groups

Legend:

SPAQ
ASAQ

The above figures showed that under treatment of both medicines, between Day7 and 14, the clinical fever and the Geometric Mean parasite Density levels increased. After Day 14, there is a sharp decrease of the clinical fever.

Both treatment groups had a mean temperature below 37˚C on day 1. Therefore, the two drugs AS+AQ and SP+AQ have a tendency to quickly lower the patient's body temperature, with SP+AQ lowering the temperature a little more than AS+AQ (figure 16).

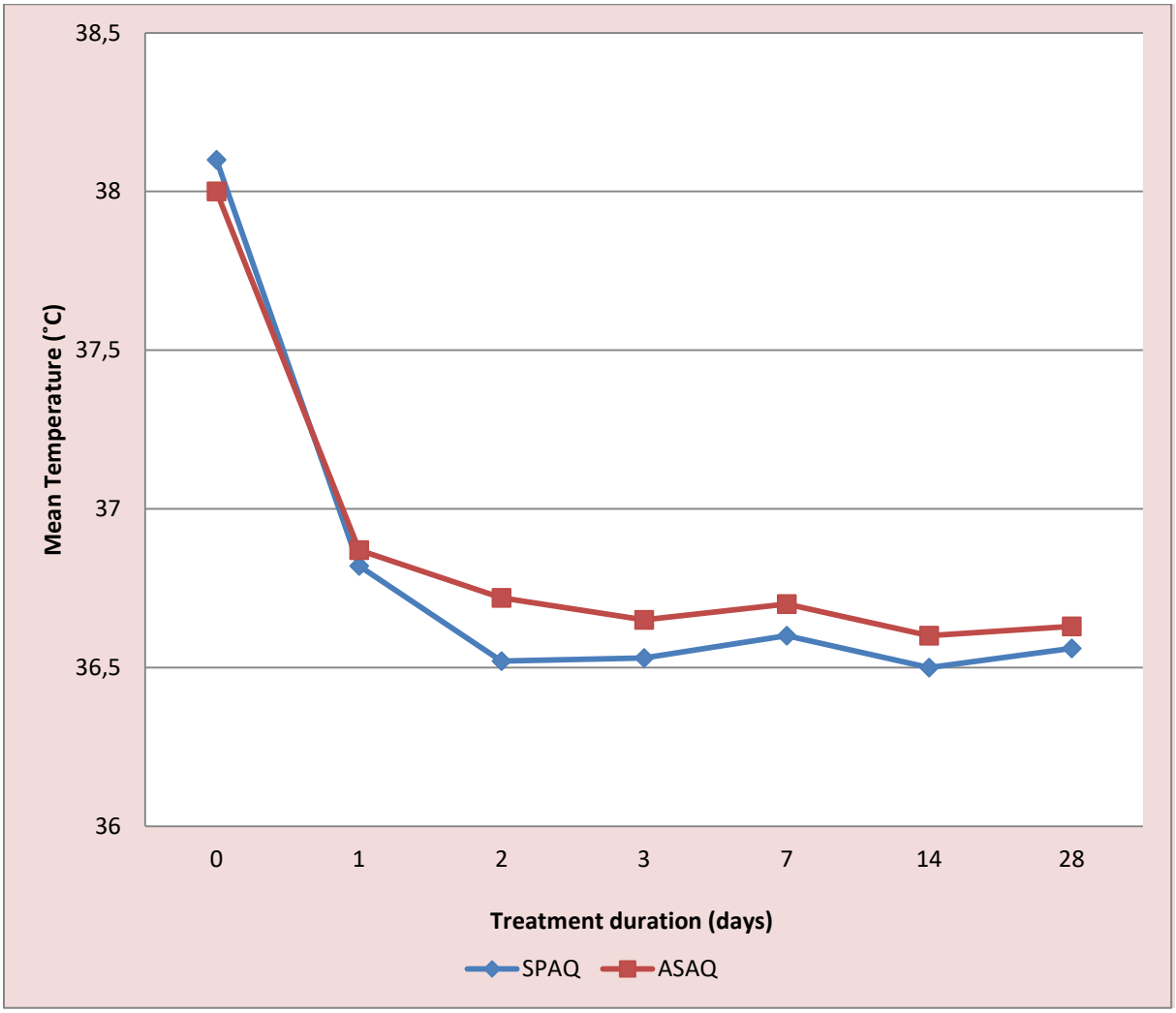


Figure 16: Mean temperature in the AS+AQ or SP+AQ treatment groups

Legend: SPAQ ASAQ

III.4.3.4. Pharmacovigilance following AS+AQ and SP+AQ Administration

Adverse events reported following AS+AQ and SP+AQ administration during the 28-day follow up period were: vomiting in 3 study participants and diarrhea in one study participant. A total of 4 adverse events were recorded with no serious adverse event. **There was a statistically significant (p value=0.001) change in the mean hemoglobin in the group of**

children who were on the SPAQ treatment group on Day 7 when compared with Day 0 (table 17).

Table 17: Hemoglobin levels on day 0 and Day 7 in the AS+AQ and SP+AQ treatment groups

TREATMENT GROUP				
Variable	***AS+AQ***		***SP+AQ***	
	Mean±SD	p-value*	Mean±SD	p-value*
HbDay 0 (g/dl)	10.2 ± 2.4	0.169	9.96 ± 2.5	0.001
HbDay 7 (g/dl)	10.5 ± 2.9		10.56 ± 2.4	
Hb D0-Hb D7 (g/dl)	-0.39		-0.6	
* paired t test				

III.4.3.5. Study Endpoints of the Study Population

At the end of the study, there were no Late Clinical and Parasitological Failures observed in both treatment groups. However, adequate clinical and parasitological response was higher on day 14 than on day 28 for both treatment groups. Finally, there were 24 (20.51%) ETF and 21 (17.80%) ETF in the AS+AQ and SP+AQ treatment groups respectively (table 18).

Table 18: Characteristics and study outcome of trial participants

Region						
	North		FarNorth		Total	
Characteristic/Outcome	AS+AQ	SP+AQ	AS+AQ	SP+AQ	AS+AQ	SP+AQ
Number enrolled	54	55	63	63	117	118
Number completed study	31	33	56	47	85	80
Withdrawals/ Loss to followup	7	9	7	4	14	13
EarlyTreatment Failure	5	7	19	14	24	21

(ETF)						
Late Clinical Failure (LCF)	0	0	0	0	0	0
Late Parasitological Failure (LPF)	0	0	0	0	0	0
ACPR* on Day 14	50(92.6 %)	54(80.0 %)	49(87.5 %)	47(100 %)	99(84.6 %)	91(77.1 %)
ACPR* on Day 28	26(83.9 %)	26(78.9 %)	37(66.1 %)	33(70.2 %)	61(71.8 %)	59(73.75 %)
ACPR = Adequate Clinical and Parasitological Response; * PCR Uncorrected cure rates						

III.4.3.6. SP+AQ and AS+AQ Impact on Malaria Morbidity

To test of equality of survival distributions for different levels of treatment code using the log rank(Mantel-Cox) to compare if the median time to parasite clearance during the 28-day follow up period was not 28 for significant (p=0.963). **The curve shows that parasite clearance was the same from day 7 to day 28 in the AS+AQ and SP+AQ drug groups, as it is presented on** figure 17.

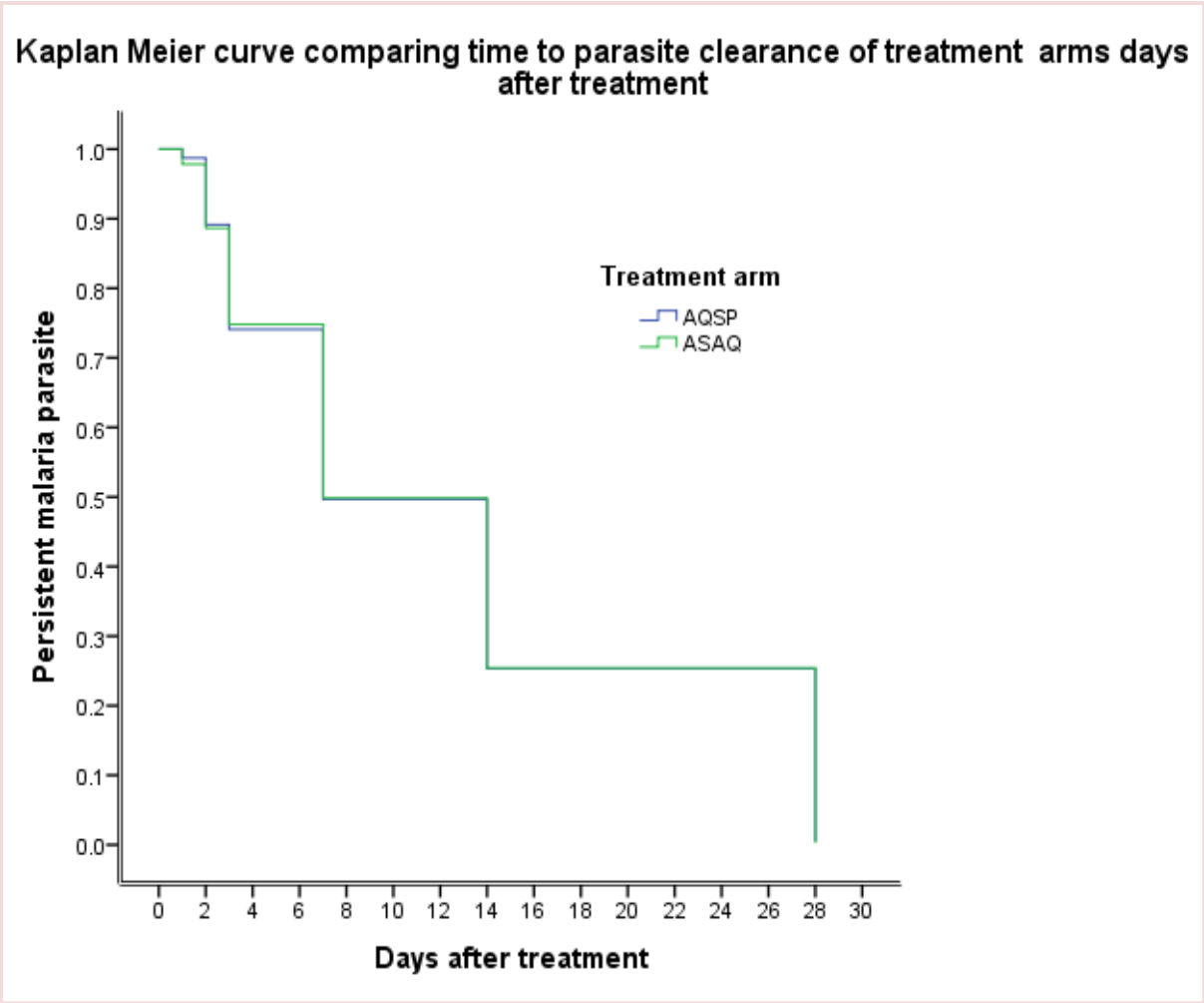


Figure 17: Kaplan Meier Survival curve in the ASAQ and SPAQ treatment groups.

III.4.3.7. Corrected PCR by *MSP2* Amplification for Cases of Treatment Failure Detected by Microscopy

In order to understand the genetic patterns of the treatment failures, the filter papers of all 45 cases of early treatment failure (24 AS+AQ group and 21 SP+AQ group) were collected for msp2 amplification (Day 0 and Day 7 for each case). This was to detect recrudescence from re-infection. This shows how sensitive the PCR diagnostic method is to false positive microscopic results (figure 18).

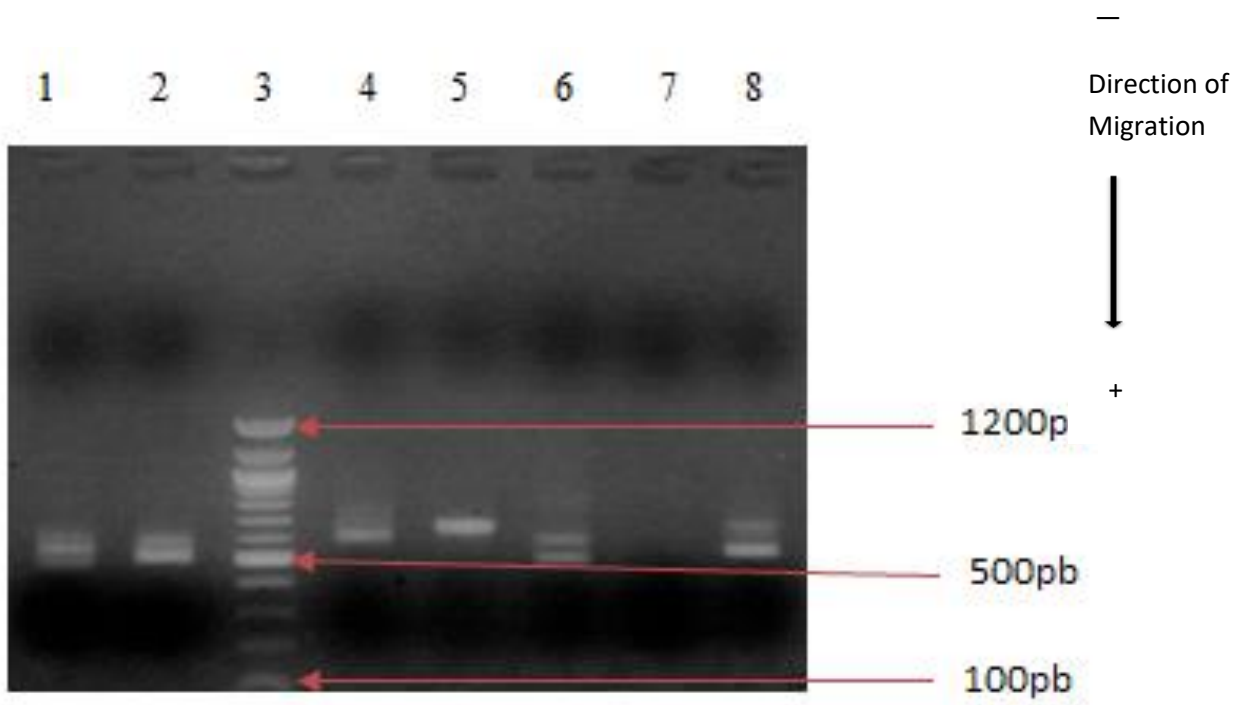


Figure 18: Electrophoregram of msp2 genotyping in cases of treatment failure

Legend: Lanes 1, 2, 4 and 5: Belonging to two patients on day 0 (Lanes 1 and 4) and day 7 (Lanes 2 and 5) showing recrudescence; Lane 3: Molecular weight marker; Lanes 6 and 7: Belonging to one patient on day 0 (Lane 6) and day 7 (Lane 7) respectively showing ACPR; Lane 8: Positive control (with known parasite DNA)

Of the 45 samples amplified, 26 were successfully genotyped for the msp2 gene. Of these 26, only 7 were confirmed recrudescent to either AS+AQ or SP+AQ. There were no cases of re-infection. This is showed on figure 19 below.

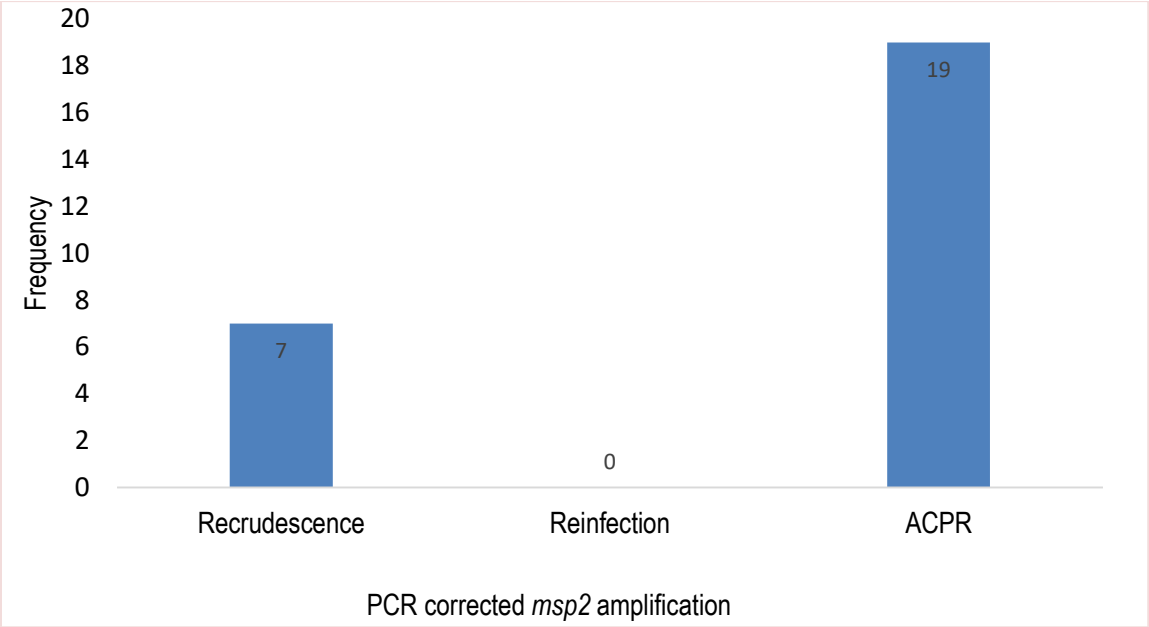


Figure 19: Recrudescence cases observed in msp2 amplification

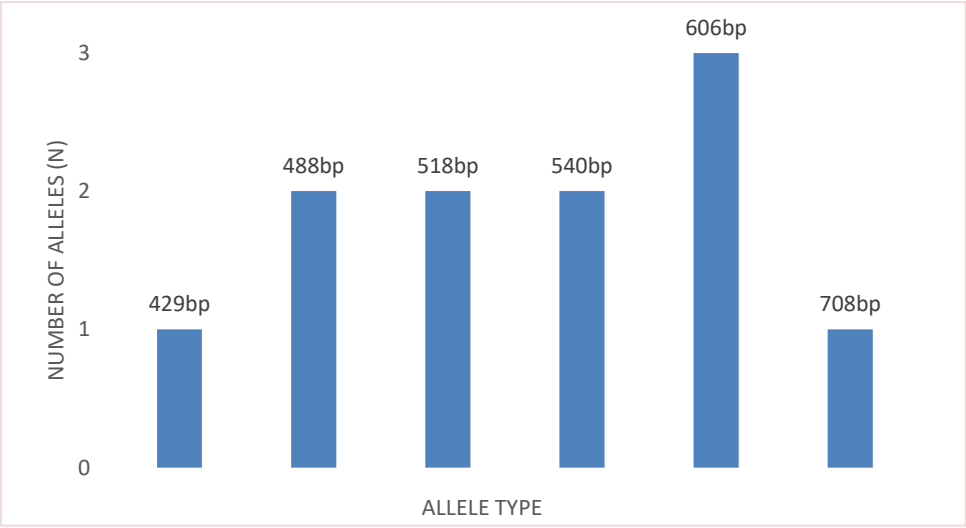


Figure 20: Number of alleles and Allele types in the recrudescence cases

The genotyping of the recrudescence cases allowed to identify 06 different types of alleles. This explain the reason why there should be different causes of recrudescences that could led to different mutation sites, responsible to resistances, as presented in figure 20.

III.4.3.8. Mutations in the dhfr gene in the PCR Corrected Cases of Treatment Failure

DNA extracts on day 0 of all the 7 identified recrudescent cases were digested with two enzymes; Xmn I and Bsr I (figure 21). These enzymes were used to identify mutations at the C59 and S108 codons of the dhfr gene respectively. The samples were compared with ACPR samples.

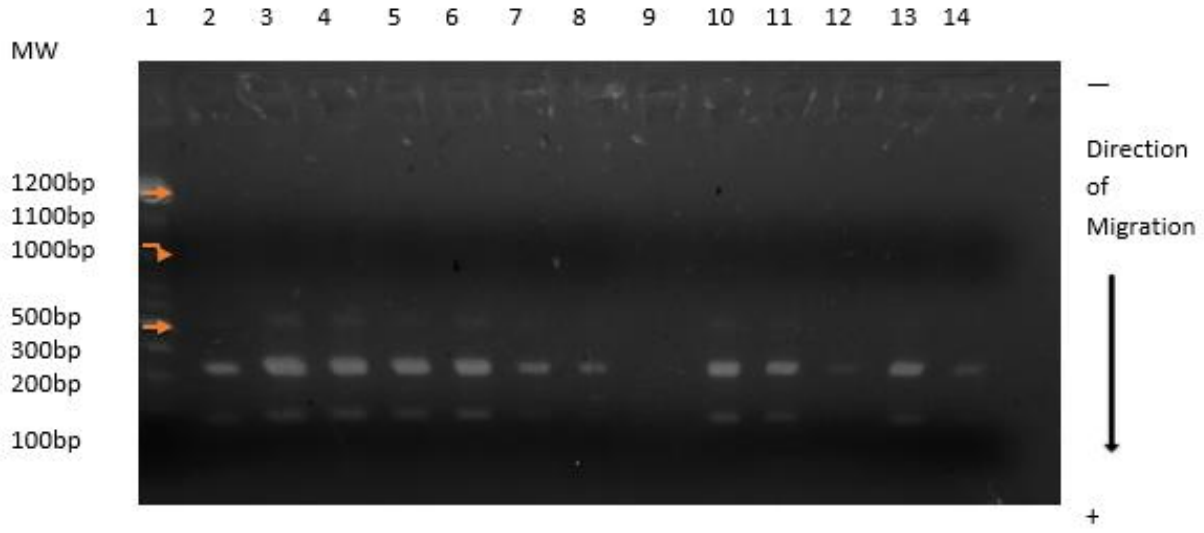


Figure 21: Electrophoregram of RFLP products after digestion with Xmn I enzyme.

Legend: Lane 1: *Molecular weight marker;* ***Lanes 2 to 8:*** *All 7 recrudescence cases showing C59R mutations (516bp wildtype; 327bp, 197bp);* ***Lanes 10 to 13:*** *4 ACPR cases showing C59R mutations;* ***Lane 9:*** *Negative control (with no DNA);* ***Lane 14:*** *Positive control (with known parasite DNA).*

The figure above showed the need to determine genotypes's cases, before the selection of the antimalarial protocol to be administered; this will avoid possibilities of toxicity observed amongst slow and rapid metabolizers.

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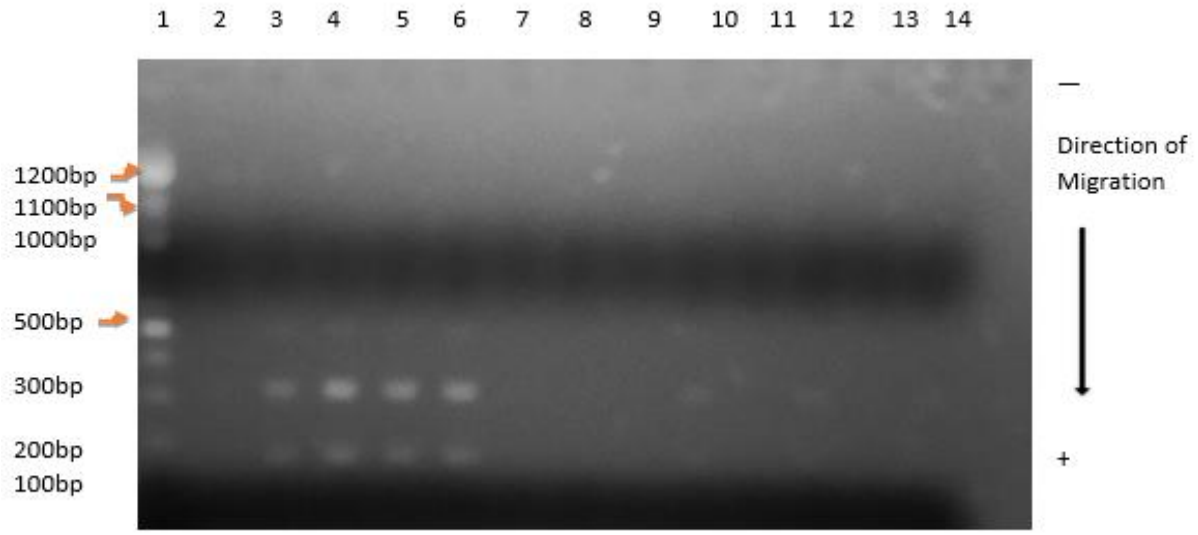


Figure 22: Electrophoresis of RFLP products after digestion with Bsr I enzyme.

Legend: Lane 1: *Molecular weight marker;* ***Lanes 2,7 and 8:****3 recrudescence cases showing no mutation on the S108 codon of the dhfr gene (506bp wildtype);* ***Lanes 3 to 6:*** *4 recrudescence cases showing S108N mutations (506bp wildtype; 310bp; 182bp);* ***Lane 9:*** *Negative control (with no DNA);* ***Lane 10:*** *Positive control (with known parasite DNA).*

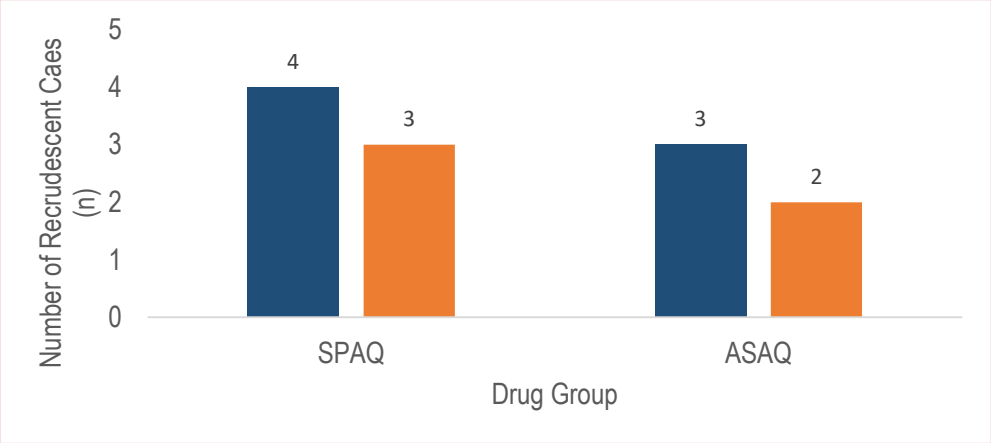


Figure 23: Frequency of mutations in the dhfr gene on recrudescence cases

Legend: C59R S108N ;

where C: Cysteine; R: Arginine; S: Serine; N: Asparagine; while 59 represents the codon of the point mutation from cysteine to arginine (mutant), and 108 the codon of the point mutation from serine to asparagine (mutant).

III.4.3.9. PCR-Corrected Cure Rates in the AS+AQ and SP+AQ Drug Groups

It was observed that the cure rate in the AS+AQ group was at 91.8% while the cure rate in SPAQ was at 91.4%. The difference was not statistically significant (p=0.435).

Table 19: Distribution of recrudescence cases according to PCR-corrected cure rates

	TREATMENT GROUP			
	AS+AQ	***SP+AQ***	***Total***	**p value**
North: Far North	2:1	3:1	5:2	0.435
Cure rate (%)	91.8%	91.4%	91.6%	
Failure rate (%)	8.2%	8.6%	8.4%	

These Distribution of recrudescence cases are presented in table 19.

III.5. Pharmacogenomic profiles of the Northern regions of Cameroon: CYP2C8 and NAT2 markers role in metabolism in the studied population

III.5.1. Characterization of the *CYP2C8* gene

III.5.1.1. Amplification of the *CYP2C8* gene

All the DNA samples that were extracted were amplified. The electrophoregram below was obtained after the amplification of the *CYP2C8* gene and shows bands between 300bp and 400bp. The plot of a standard curve of distances between the different bands of the molecular weight markers (cm) against log of molecular weights (Log MW) represented below permitted to calculate the weight of the bands observed (figure 24).

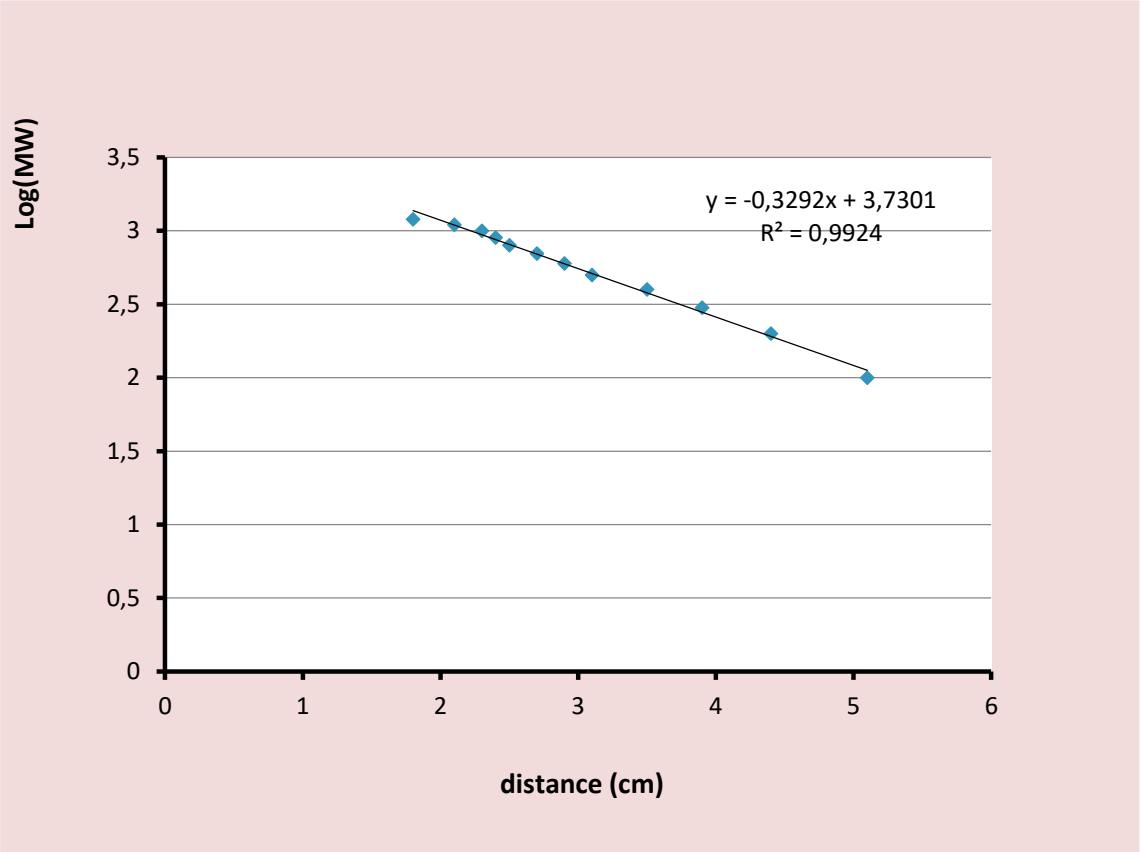


Figure 24: Standard curve for the estimation of band sizes obtained after electrophoresis

All the bands were around 312bp as expected (figire 25).

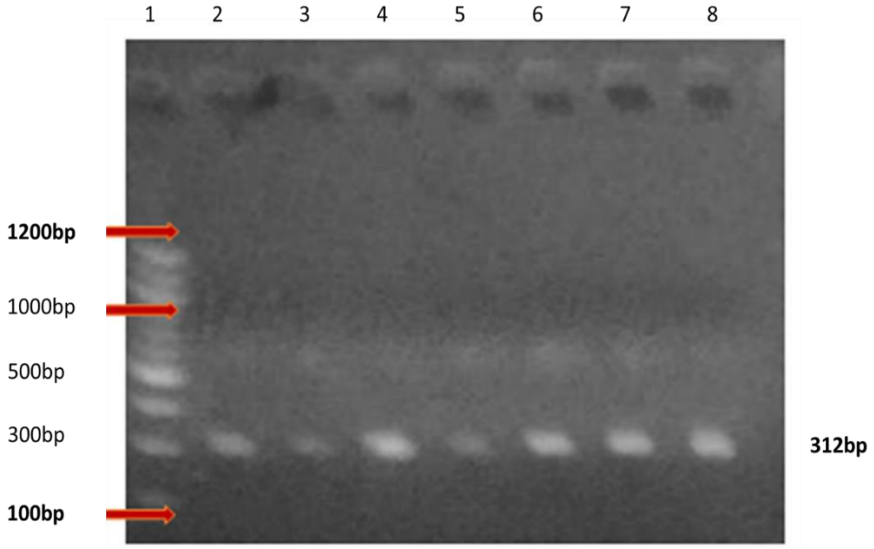
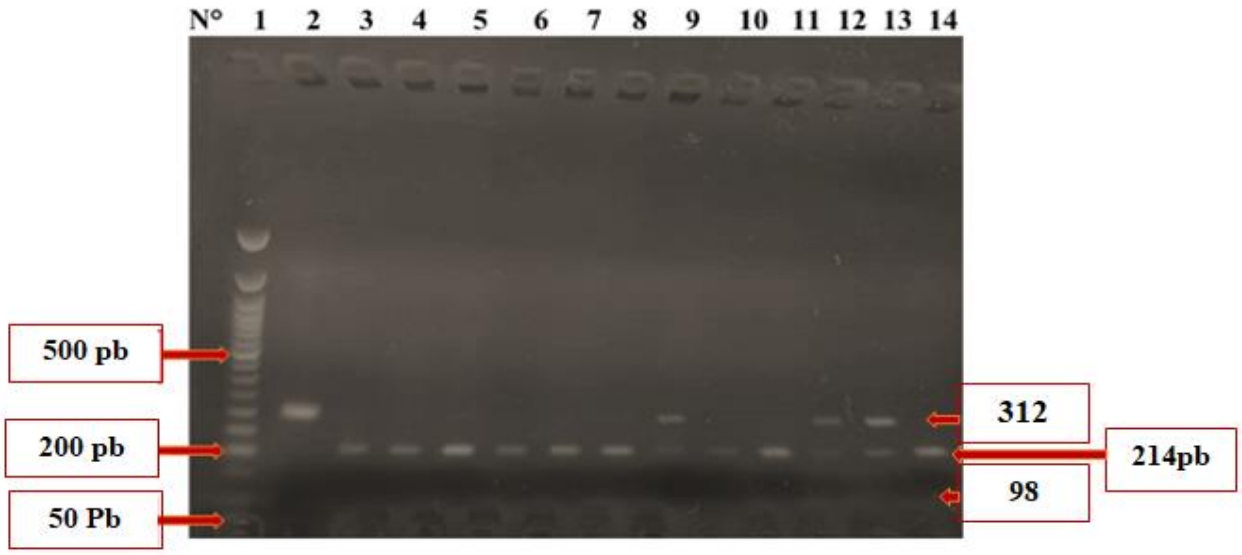


Figure 25: Electrophoresis from amplicons of CYP2C8 gene

Legend: N°1 = Molecular weight Marker. N°2, 3, 4, 5, 6, 7, 8 = patients.

III.5.1.2. Enzymatic digestion of the CYP2C8 amplicons with BclI restriction enzymes

After amplification of a 312bp of the *CYP2C8* gene, the amplicons were then digested with BclI restriction enzymes, in order to identify the different genotypes of this portion of the gene. The electrophoregram in Figure 26 below shows the distances of migration of the different digests. Their sizes were estimated as described earlier, using the linear regression curve of equation Log10 (MW) = f (d). At the end of this, bands at 98, 214 and 312 bp were observed.



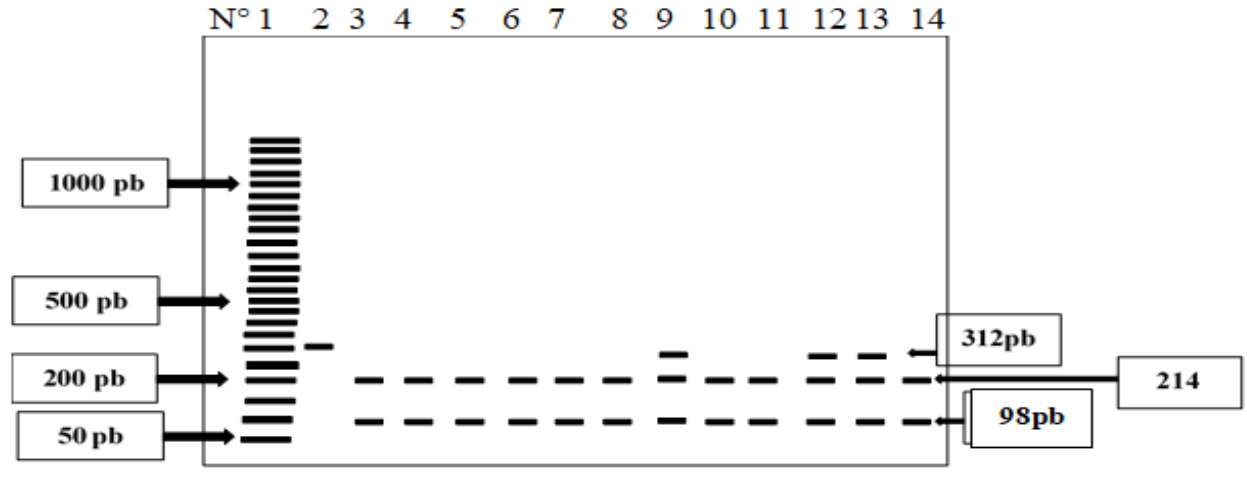


Figure 26: Electrophoresis profile of gene CYP2C8 digestion with BclI Enzyme

Legend : *N°1 = Molecular weigth Marker; N°2 = Undigested control sample with an area of 312 pb ; N°3, 4, 5, 6, 7, 8, 10, 11, 14 show areas of 214 and 98 pb (CYP2C8*1/1) ; N°9, 12,13 show areas of 312, 214 et 98 pb (CYP2C8*1/2)*

III.5.1.3. Allelic frequency of the CYP2C8 gene

The CYP2C8*1 and CYP2C8*2 alleles were found, with allele CYP2C8*1 being predominant (76%) and alleles CYP2C8*2 less found (24%) in the study population (Figure 27).

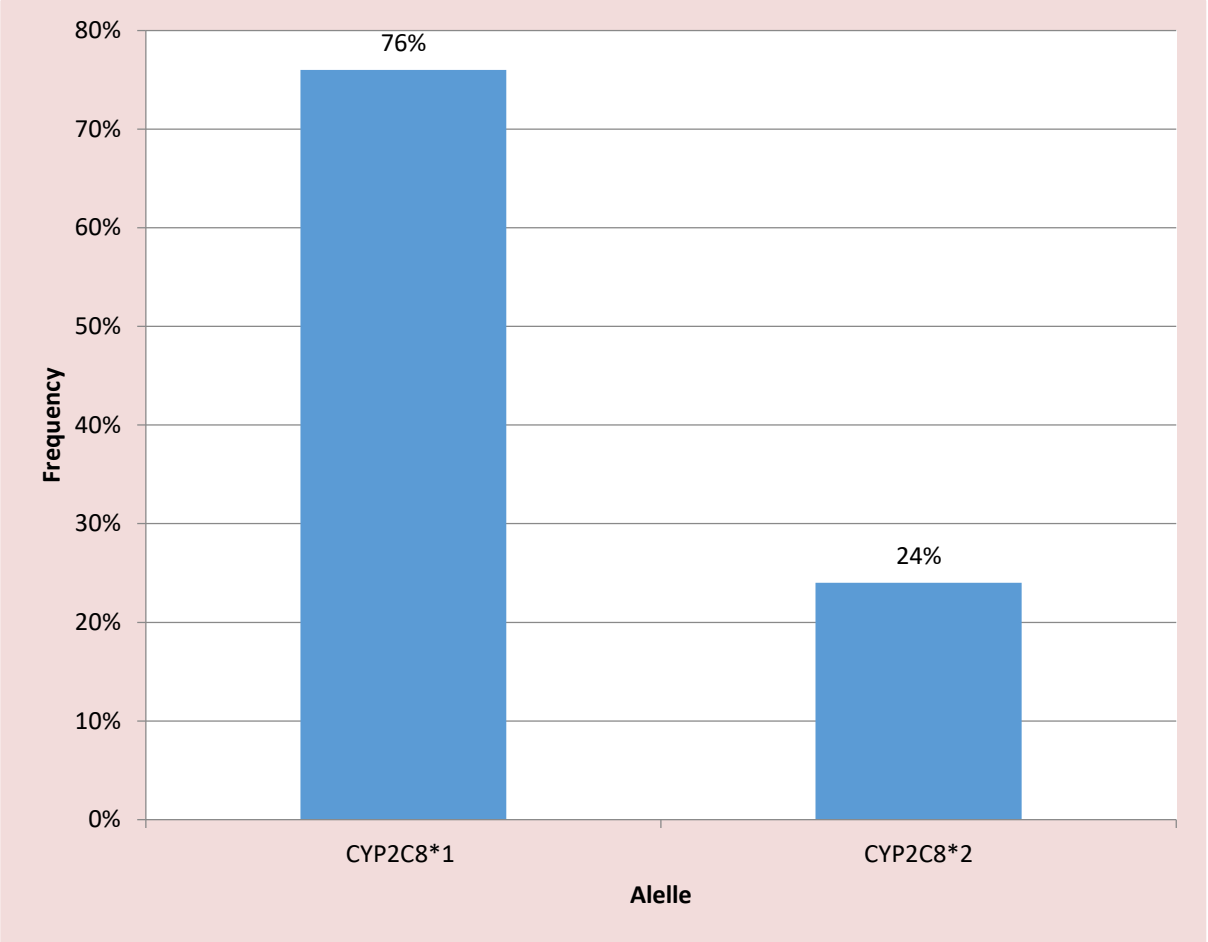


Figure 27: Distribution of allelic gene CYP2C8

III.5.1.4. Genotypic (phenotypic) frequency of the CYP2C8 gene

The fast (CYP2C8*1/1) and slow (CYP2C8*2/2) metabolizers phenotypes were found to be homozygous; while intermediate (CYP2C8*1/2) metabolizers phenotypes were found to be heterozygous. Fast metabolizers being predominant (70%) and slow metabolizers phenotypes less found (3.3%) in the study population (Figure 28).

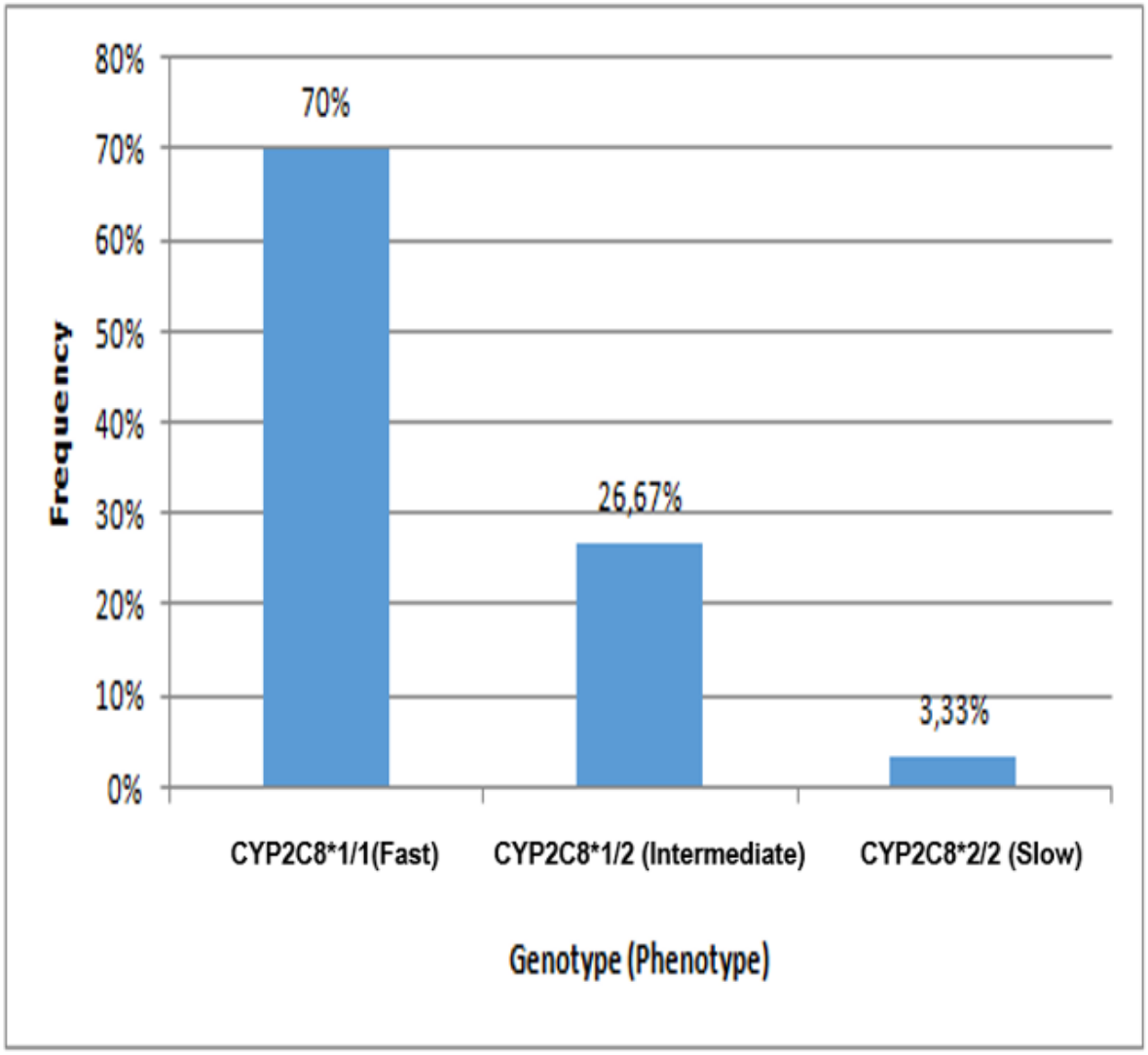


Figure 28: Frequency of CYP2C8 genotype and phenotypes among study participants

III.5.1.5. Association between CPY2C8*2 phenotypes and treatment outcomes

According to the different phenotypes (Fast, Intermediate and Slow metabolizer) from the CYP2C8 gene, the risk to develop Early treatment failure (ETF) was calculated by binary Logistic regression. The Intermediate metabolizers phenotype was at risk of developing ETF (P=0.000) in this study population (table 20).

Table 20: Association between CPY2C8*2 phenotypes and treatment outcomes

Phenotype	No. examined (%)	Treatment outcomes (%)			Level of significance
		ETF	LPF	ACPR	
Slow	3 (3.3)	0 (0.0)	3 (100.0)	0 (0.0)	X^2=174.902 P=0.000
Intermediate	24 (26.7)	23 (95.8)	0 (0.0)	1 (4.2)	
Fast	63 (70.0)	0 (0.0)	0 (0.0)	63 (100.0)	

ETF: Early treatment failure; LPF: Late parasitological failure; ACPR: Adequate clinical and parasitological response

The fast metabolizers phenotype showed adequate clinical and parasitological response (ACPR) at 63%.

III.5.2. Analysis of NAT2 gene SNPs

III.5.2.1. Amplification of *NAT2* gene

The electrophoregram below of PCR products was obtained after the amplification of the *NAT2* gene and shows bands between 500bp and 600bp, exactly at 535bp (base pairs) for all patients, which characterize the presence of the NAT2 gene (figure 29).

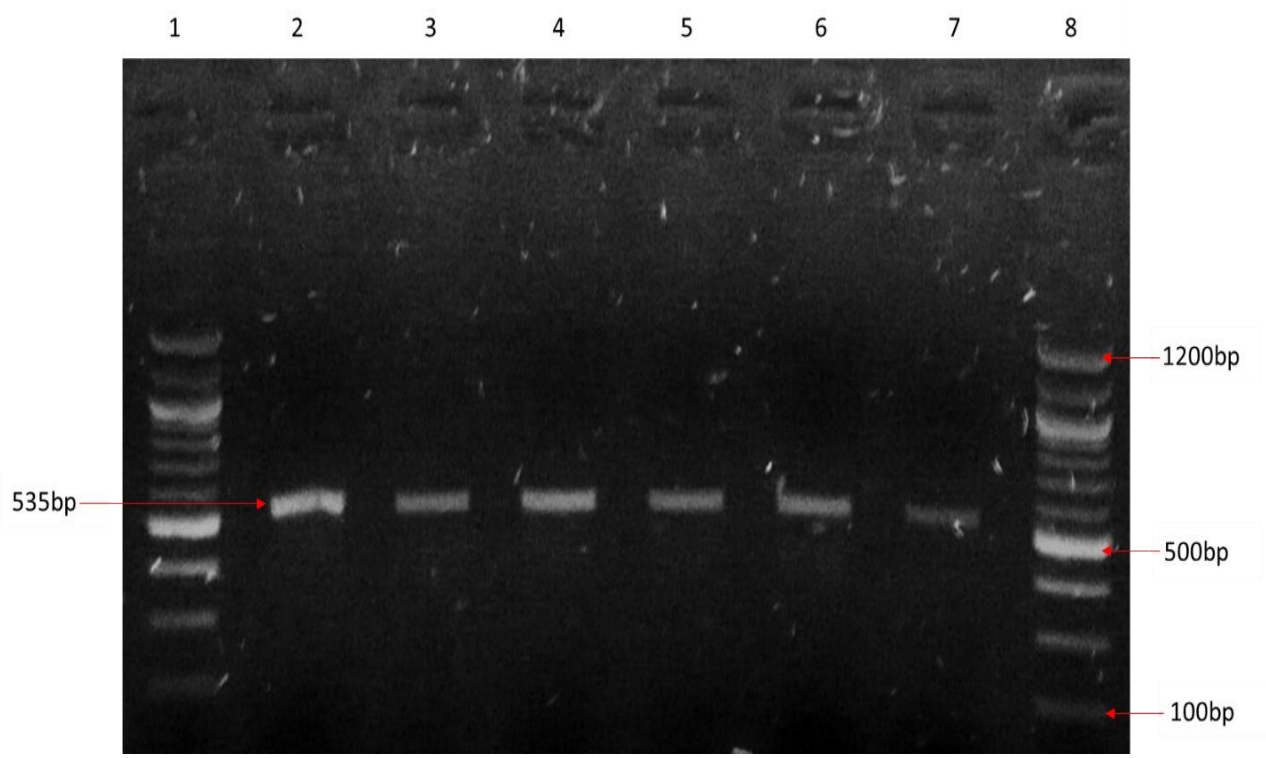


Figure 29: Profile of PCR products of NAT2 gene

Legend: *535bp in ligne 2, 3, 4, 5, 6, 7 represent the present of NAT2 gene, molecular weight marker (1 and 8).*

III.5.2.2. Digestion of NAT2 amplicons with BamHI, KpnI and TaqI restriction enzymes

After amplification of a 535bp of the *NAT2* gene, the amplicons were then digested with BamHI, KpnI and TaqI restriction enzymes, in order to identify the different genotypes of this portion of the gene. The electrophoregram in Figures 30 below shows the distances of migration of the different digests. Their sizes were estimated as described earlier, using the linear regression curve of equation Log10 (MW) = f(d). The different genotypes were determined as described by the literature.

After digestion with Kpn1,

The electrophoregram of products of digestion obtained after digestion with Kpn1 revealed; bands at 535bp indicating the presence of homozygotes with two NAT2*5 alleles (NAT2*5/5), bands at 535bp and 483bp indicating the presence of heterozygotes with only one NAT2*5 alleles, bands at 483bp indicating the absence of the NAT2*5 allele.

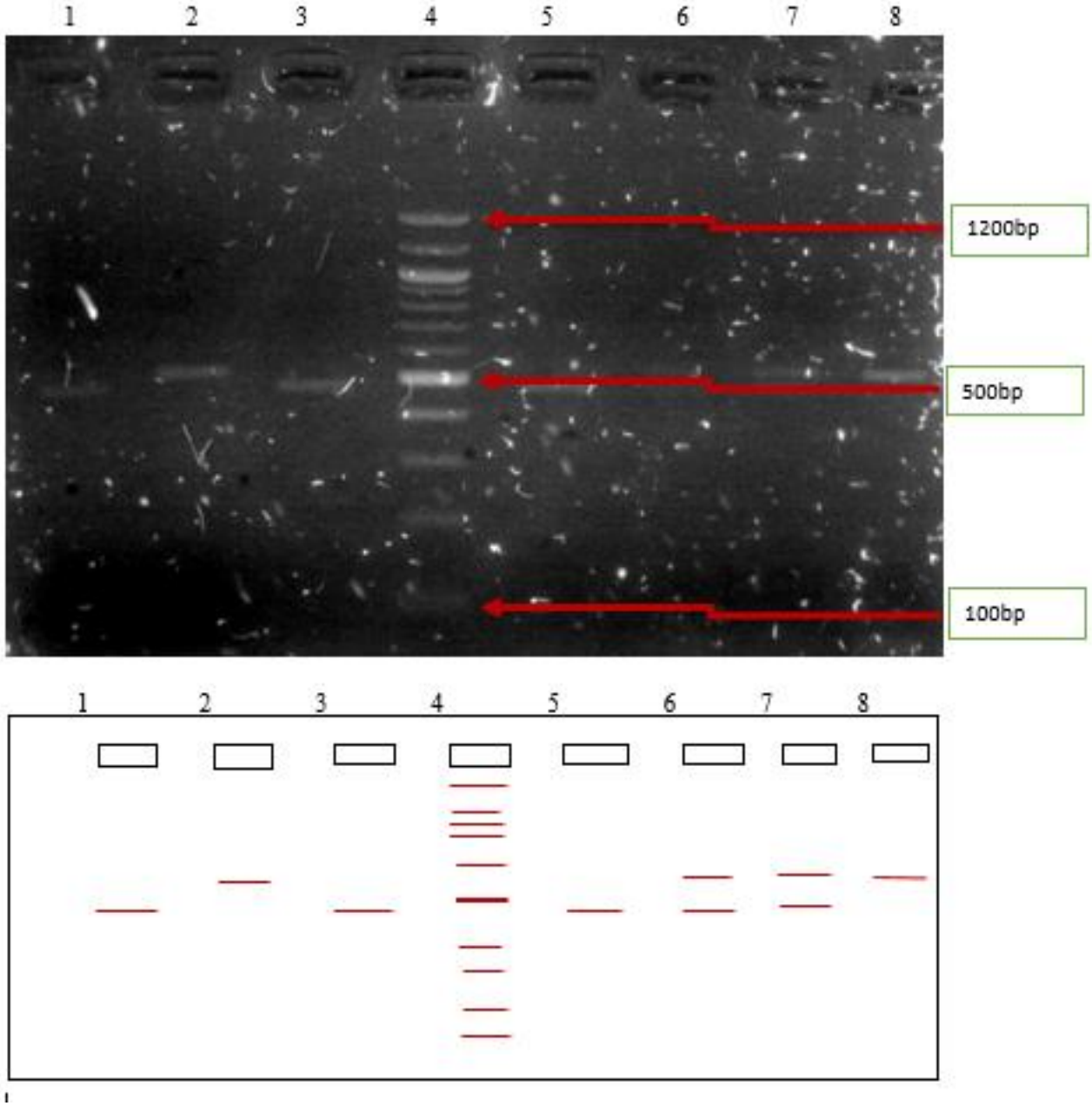


Figure 30: Digestion profile of NAT2 gene with Kpn1

Legend: *535bp in ligne 2 represent patient with NAT2*5/5, 483bp in ligne 1,3,5 represent patients without NAT2*5, 535bp and 483bp in ligne 6 and 7represent patients with NAT2-*/5, undigested (8) and molecular weight marker (4).*

After digestion with Taq1

The electrophoregram of product of digestion after digestion with Taq1 revealed: bands at 330bp and 205bp indicating the presence of homozygotes with two NAT2*6 alleles (NAT2*6/6), bands at 330bp, 205bp, 170bp, and 160bp indicating the presence of heterozygotes with just one NAT2*6 allele, bands at 205bp, 170bp and 160bp indicating the absence of NAT2*6 (figure 31).

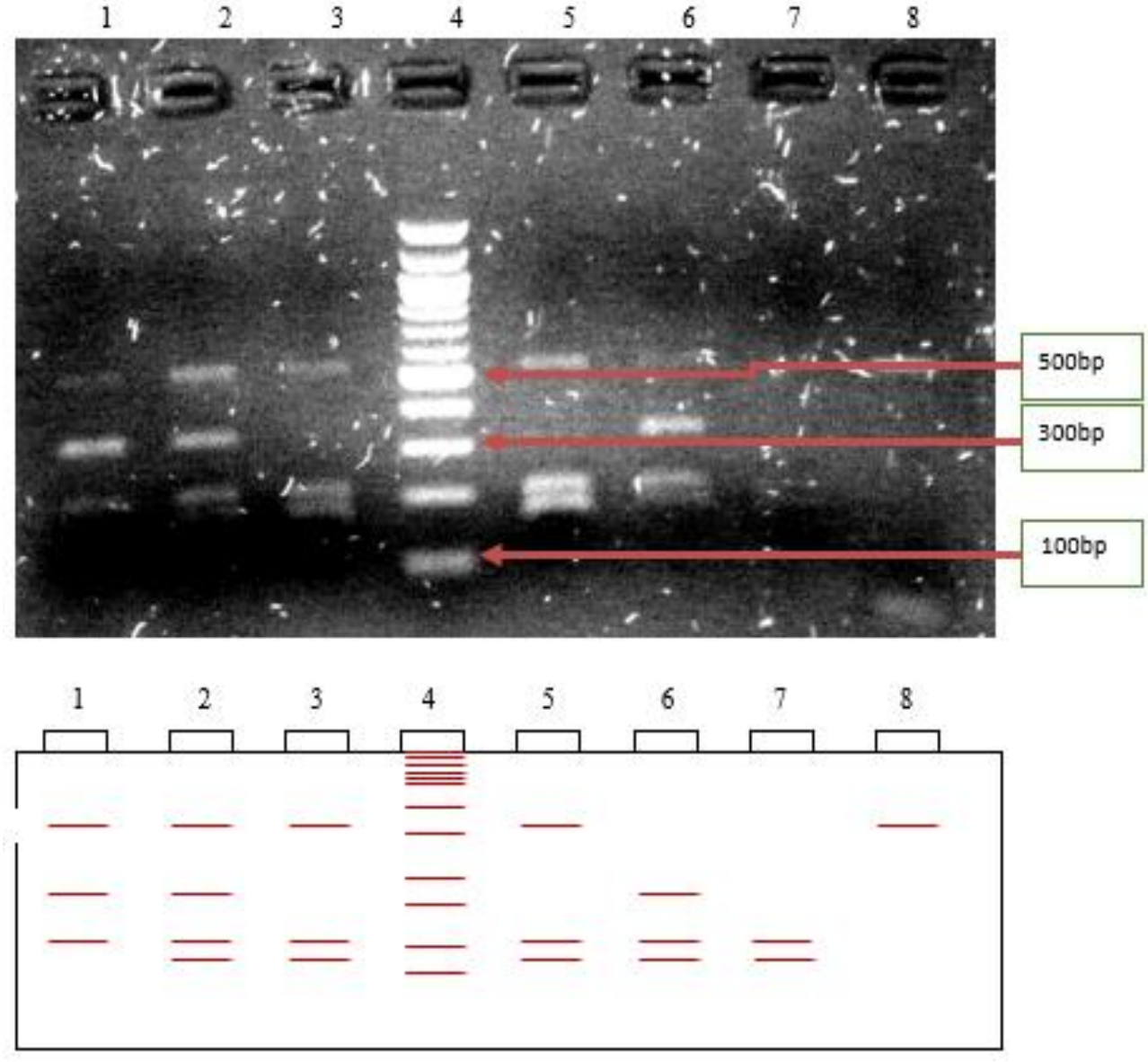


Figure 31: Digestion profile of NAT2 gene with Taq1
Legende:*535bp, 330bp and 205bp in ligne 1 represent patient with NAT2*6/6, 535bp, 330bp, 205bp and 170bp in ligne 2 and 6 represent patient with NAT2*+/6, 535bp, 205bp and 170bp in ligne 3,5 and 7 represent patient without NAT2*6, undigested (8), molecular weight marker (4)*

After digestion with BamH1

Finally, those digested with BamHI revealed: bands at 535bp and 428bp indicating heterozygotes with just one NAT2*7 allele, bands at 428bp indicating the absence of NAT2*7 allele (Figure 32).

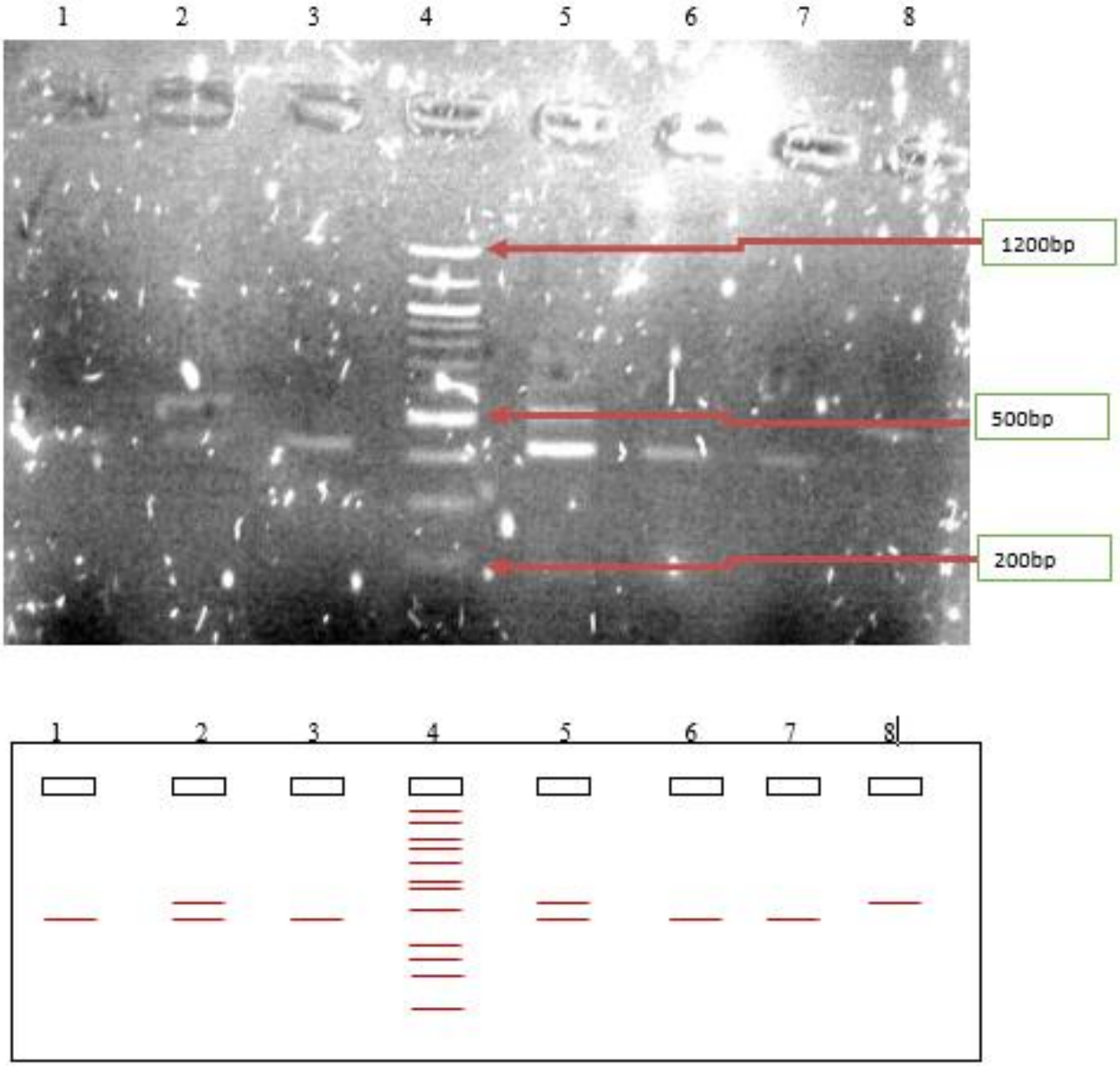


Figure 32: *Digestion profile of NAT2 gene with BamH1*

Legende: *535bp and 428bp in ligne 2 and 5represent patient with NAT2*+/7; 428bp in ligne 1, 3, 6 and 7 represent patient without NAT2*7, undigested (8), molecular weight marker (4)*

After total digestion of the NAT2 gene using the three restriction enzymes: BamH1, Kpn1 and Taq1, we noted the total absence of NAT2*5, NAT2*6 and NAT2*7, which automatically confirms the presence of NAT2*4 (NAT2*4/4), as illustrated in patient 3 above.

In the case of digestion of only one allele of the NAT2 gene using the aforementioned enzymes, we note the absence on NAT2*5, NAT2*6 and NAT2*7 on one allele and replaced by NAT2*4. This is illustrated in patient 7 above.

The bands obtained after digestion with the restriction enzymes: BamHI, KpnI and TaqI of PCR products of each patient were combined in order to determine their individual genotypes as shown below (Table 21).

Table 21: Band lengths after digestion per restriction enzyme with respect to genotypes

BamH1 bands (bp)	**Kpn1 bands (bp)**	**Taq1 bands (bp)**	**Genotypes**
428	535	205 ; 170	NAT2*5/5
428	535 ; 483	205 ; 170	NAT2*4/5
428	535 ; 483	330 ; 205 ; 170	NAT2*5/6
535 ; 428	535 ; 483	205 ; 170	NAT2*5/7
535 ; 428	483	205 ; 170	NAT2*4/7
535 ; 428	483	330 ; 205 ; 170	NAT2*6/7
428	483	330 ; 205	NAT2*6/6
428	483	330 ; 205 ; 170	NAT2*4/6
428	483	205 ; 170	NAT2*4/4

III.5.2.3. Allelic frequencies of NAT2 in Garoua

The NAT2*4, NAT2*5, NAT2*6 and NAT2*7 alleles were found, with NAT2*5 being predominant (39%) and allele NAT2*7 less foun0d (4%) in the study population from Garoua (Figure 33).

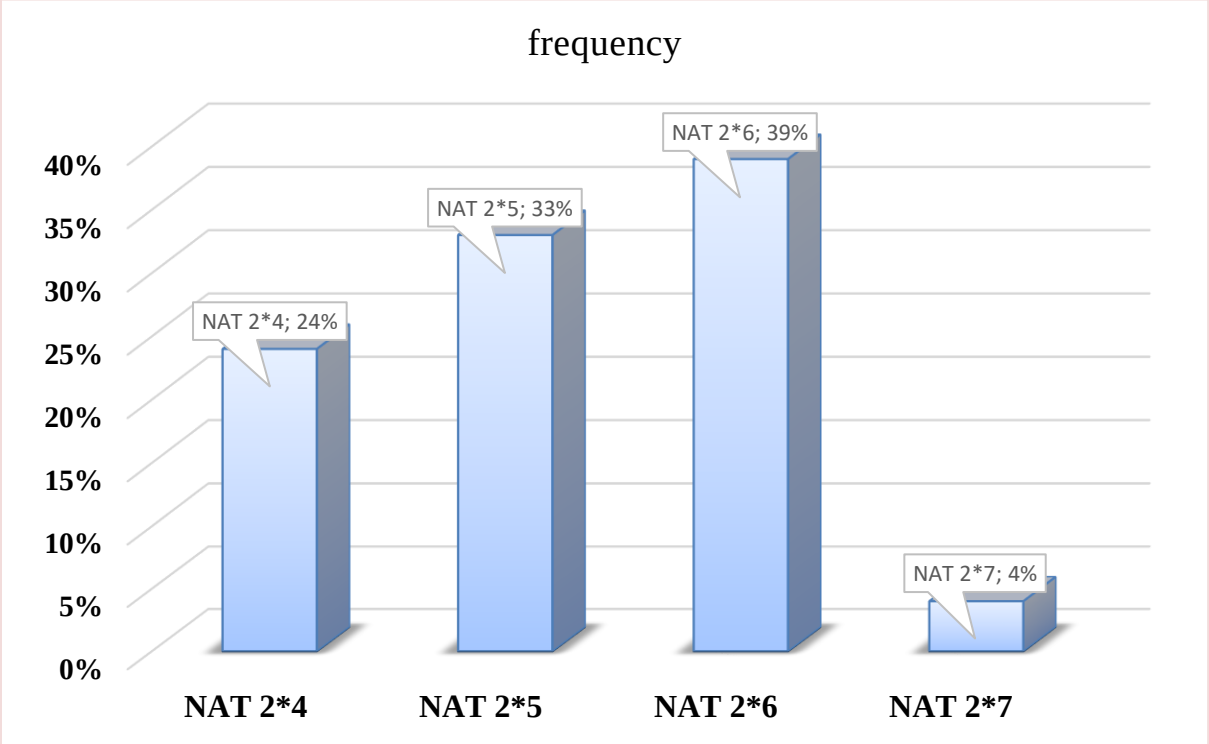


Figure 33: Allelic frequency of NAT2 in Garoua

III.5.2.4. Genotypic frequencies of NAT2 gene in Garoua

All the total DNA samples from Garoua that were extracted and amplified were digested for the genotyping. As shown by Figure 34 below the NAT2*4/4, NAT2*4/5, NAT2*4/6, NAT2*4/7, NAT2*5/5, NAT2*5/6, NAT2*5/7, NAT2*6/6 and NAT2*6/7 genotypes were found, with NAT2*5/6 being predominant (28.12%).

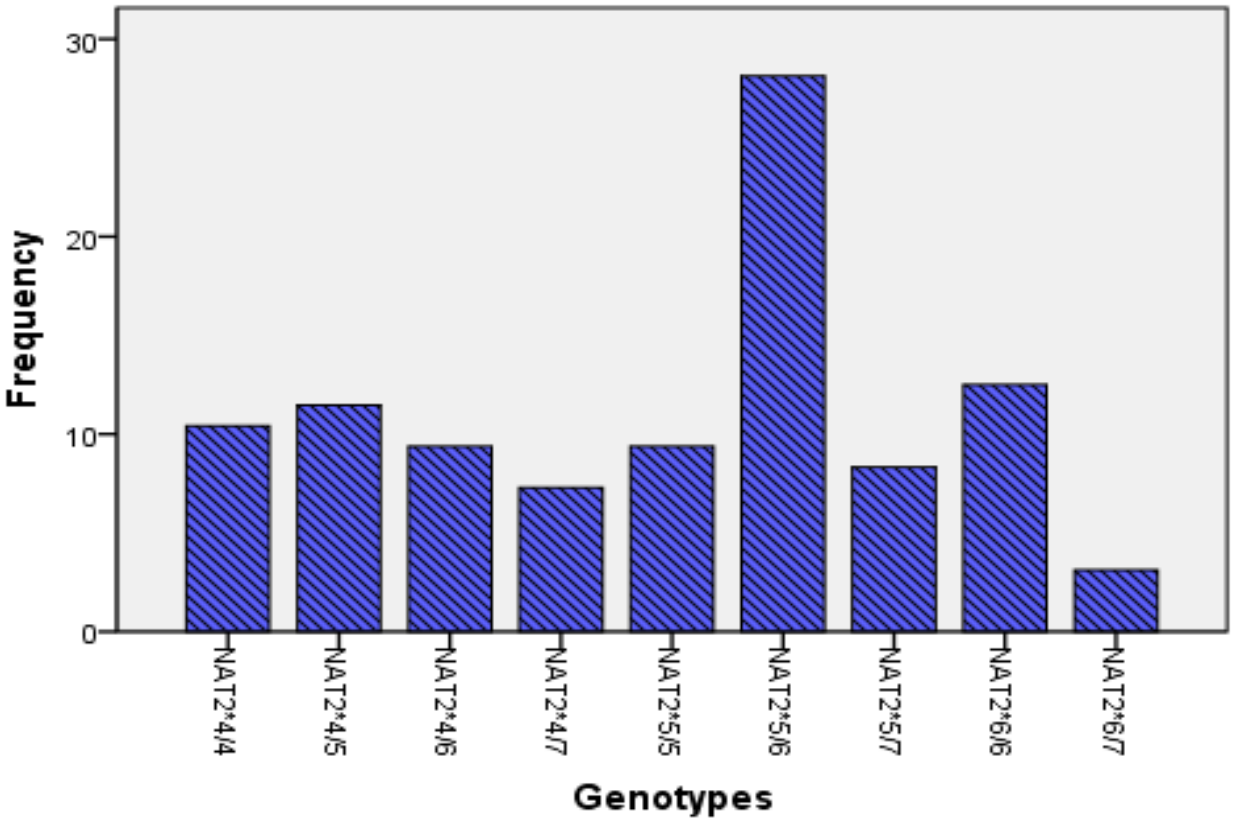


Figure 34: Genotypic frequencies of NAT2 gene in Garoua.

III.5.2.5. Phenotypic frequencies of NAT2 gene in Garoua.

The fast, intermediate and slow acetylators phenotypes were found, with slow metabolizer being predominant (61.4%) and fast metabolizer phenotype less found (10.4%) in the study population (Figure 35).

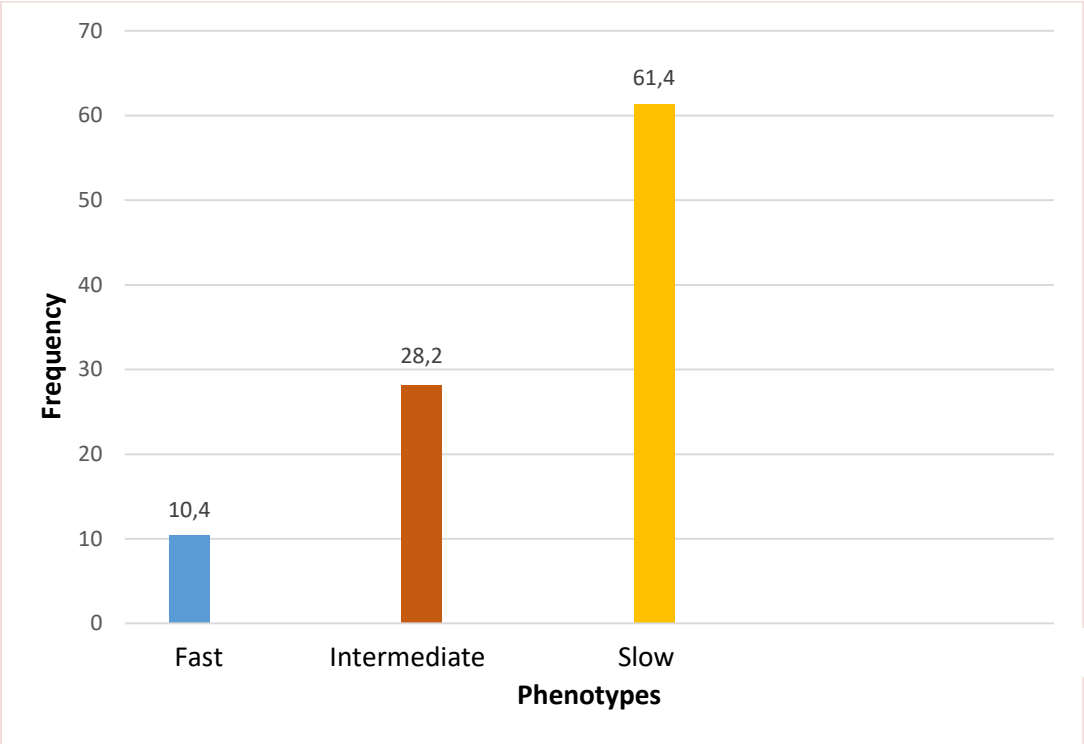


Figure 35: Phenotypic frequencies of NAT2 gene in Garoua

III.5.2.6. Analysis of treatment outcomes.

Treatment outcomes were classified according to the WHO guidelines for areas of intense transmission as adequate clinical and parasitological response (ACPR), early treatment failure (ETF), and late parasitological failure (LPF).

ACPR being the absence of parasitaemia during the follow-up period up to day 28 irrespective of axillary temperature, ETF being the presence of parasitaemia on day 3 > 25% of count on day 0 or parasitaemia on day 3 with axillary temperature > 37.5°C, and LPF being the presence of parasitaemia from day 7-28.

ACPR was much more dominant (63.5%), with respect to ETF (24%) and LPF (12.5%).

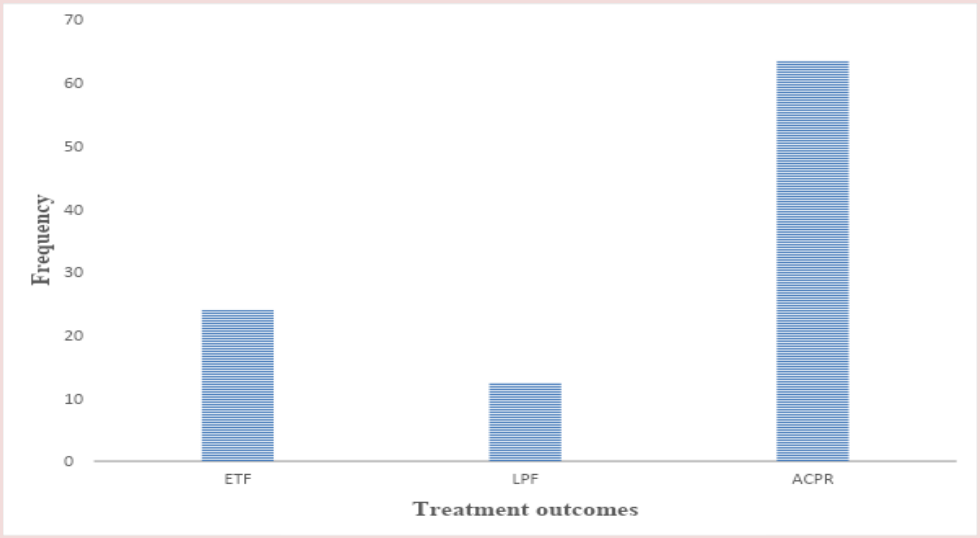


Figure 36: Frequency of various treatment outcomes.

III.5.2.7. Relationship between Phenotypes and treatment outcomes.

III.5.2.7.1. Early treatment failure and Phenotypes.

According to the different phenotypes (Fast, Intermediate and Slow acetylator) from the NAT2 gene, the susceptibility to experience early treatment failures was calculated by binary Logistic regression. The fast phenotype was much more susceptible to experience early treatment failures (OR=4.792); this association was statistically significant at the level of 0.05 (p=0.02).

Table 22: Relationship between ETF and Phenotypes

Phenotype	**EARLY TREATMENT FAILURE**				
	Present	***Absent***	***OR***	***95% CI***	***P-value***
Slow	2	19	0.271	0.058-1.265	0.08
Fast	5	4	4.792*	1.166-19.694	0.02*
Intermediate	16	50	1.051	0.381-2.905	0.923

OR: Odd's Ratio, CI: Confidence interval, (*) = statistically significant.

III.5.2.7.2: Late Parasitological failure and Phenotypes.

According to the different phenotypes (Fast, Intermediate and Slow metabolizer) from the NAT2 gene, the susceptibility to experience late parasitological failures was calculated by binary Logistic regression. The fast phenotype was much more vulnerable to experience late parasitological failures (OR=4.333); this association was statistically significant at the level of 0.05 (p=0.047).

Table 23: Relationship between LPF and Phenotypes

Phenotype	**LATE PARASITOLOGICAL FAILURE**				
	Present	***Absent***	***OR***	***95% CI***	***P-value***
Slow	2	19	0.684	0.138-3.395	0.641
Fast	6	3	4.333*	0.921-20.379	0.047*
Intermediate	7	59	0.593	0.172-2.048	0.405

OR: Odd's Ratio, CI: Confidence interval, (*) =statistically significant.

III.5.3. Cladogram

The cladogram presents three clusters:

- Cluster A with patients who are intermediate metabolizers, without pfDHFR S108N and pfDHPS C58R, resistance markers,
- Cluster C with rapid metabolizers, without the 02 resistance markers,
- Cluster B with slow metabolizers and with the 2 resistancemarkers.

It appears that slow metabolizers are at risk to develop resistance mutations than patients who are rapid and intermediates metabolizers.

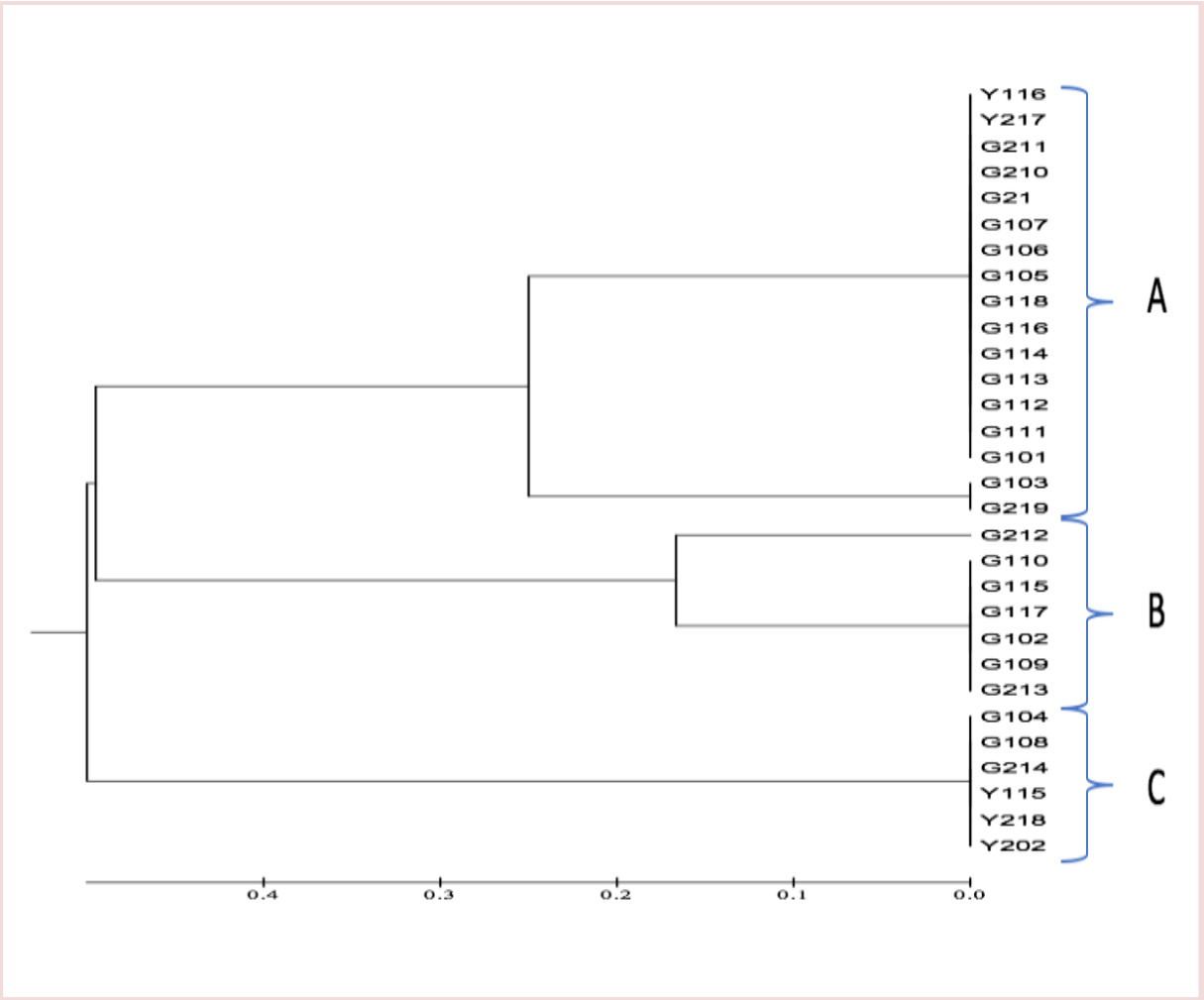


Figure 37: Cladogram of patients phenotypic statuts according to NAT 2, pfDHFR and pfDHPS genes.

Légende: A= Intermediate metabolizers without resistance polymorphisms; B= Slow metabolizers with resistance polymorphisms; C= Fast metabolizers without resistance polymorphisms

III.5.4. Protein-protein interactions network.

Some 10 proteins were identified (NAT1, CYP1A1, CYP1A2, CYP2A6, CYP2D6, CYP2E1, GSTT1, GSTM1, XDH, C21 and f33) to interact with the NAT2 protein resulting in a network diagram with 11 nodes (gene/proteins) including the NAT2 node and with 36 direct edges or interactions (figure 38). By these interactions, the NAT2 protein could influence the action of these 32 proteins and the processes in which they are implicated and vice versa.

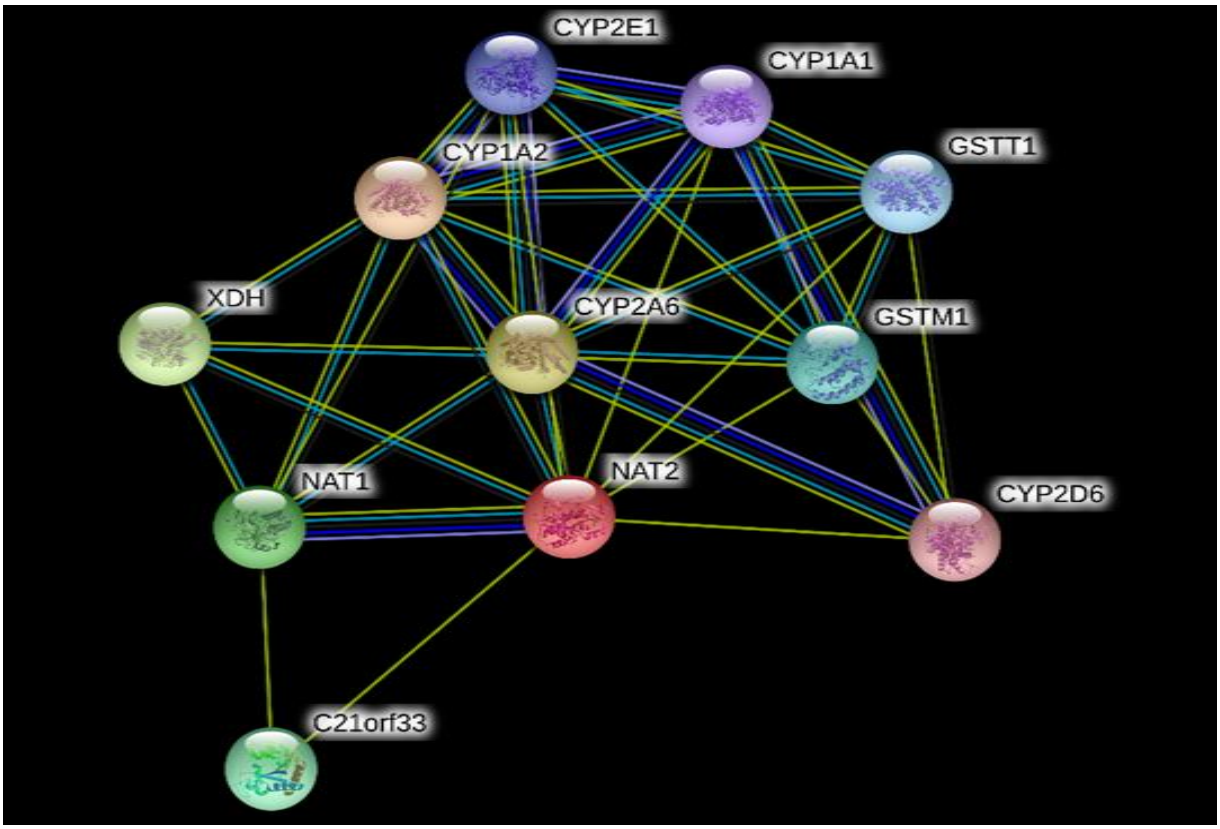


Figure 38: NAT2 protein network (protein-protein interactions).

The most important proteins and their functions in this network are: ***CYP1A1*** *(It oxidizes a variety of structurally unrelated compounds, including steroids, fatty acids, and xenobiotics);* ***CYP1A2*** *(It oxidizes a variety of structurally unrelated compounds, including steroids, fatty acids, xenobiotics, caffeine and acetaminophen);* ***CYP2A6*** *(Exhibits a high coumarin 7 - hydroxylase activity and can act in the hydroxylation of the anti-cancer drugs; cyclophosphamide and ifosphamide);* ***CYP2D6*** *(Metabolism of drugs such as antiarrhythmics, adrenoceptor antagonists, and tricyclic antidepressants);* ***CYP2E1*** *(Inactivates a number of drugs and xenobiotics);* ***NAT1*** *(Participates in the detoxification of a plethora of hydrazine and arylamine drugs);* ***GSTT1*** *(Glutathione S-transferase theta1), (Conjugation of reduced glutathione to a wide number of exogenous and endogenous hydrophobic electrophiles).*

CHAPTER IV :

DISCUSSIONS

The present study aimed to assess the role of pharmacogenomic factors in treatment outcomes among individuals with uncomplicated malaria, pharmacology and health impact of some antimalarial medicines (resistance markers) in the Northern Regions of Cameroon. In other to achieve that, we analyzed the situation of the irrational use, the presence and extent of substandard/ falsified/ counterfeit antimalaria drugs in Cameroon; in addition we investigated the antimalaria efficacy of SP+AQ versus AS+AQ in the Northern Regions, with emphasis on clinical trials analysis and treatment outcomes. Finally, we determined the pharmacogenomic profile of trial patients from Northern Regions of Cameroon by using CYP2C8 and NAT2 gene polyorphisms involved in the antimalaria drug metabolism, including their impact in treatment outcomes.

Artemisinin combination therapy (ACT) was used to understand the gaps in the knowledge and practice of providers in Africa: Case of Nigeria and Cameroon. These ACTs are first-line treatment for uncomplicated malaria in Cameroon since 2004 and in Nigeria since 2005, although many febrile patients receive less effective drugs. Patients often rely on providers to select treatment. Providers' adherence to treatment guidelines was examined using data collected from Cameroon and Nigeria from public and mission establishments, pharmacies and drug stores. The results showed that there was a gap between providers 'knowledge and practice in countries, as providers' decision to provide ACT was not significantly related to knowledge of first-line antimalarial.

Providers were, however, more likely to supply a drug if it was what they prefer, what they perceived their patients prefer or could afford, as well as information about their symptoms, previous treatment, the type of outlet, and availability of such a drug. Interventions to improve adherence to treatment guidelines should emphasize the recommended drug for all patients with the disease. It turned out that the medical delegates who advertise for pharmaceutical companies are of a great influence to their practice. According to WHO, about 100000 deaths per year in Africa are linked to counterfeit drugs. The detection of fake drugs has become more difficult over the years because of the counterfeiter’s increased ability to reproduce his own. The problems of counterfeit drugs are known to exist in both developed and developing countries. However, the extent of the problem is not really known since no global studies have been carried out. Because the ingredients of fake drugs includes chalk and paint which could lead to poisoning and result in death, the distribution of fake falsified and substandard drugs has tragic consequences and is equivalent to murder. In the studies that were done involving

the WHO when they investigated the quality of the medication in Sub Saharan Africa, it was found that between 40% and 60% were fake medications or that these did not have the right amount of active ingredient (WHO, 2013).

These substandard/falsified/counterfeit antimalaria medicines increase the risk of artemisinin resistance developed by the use of sub therapeutic dosages of antimalarials. For decades, counterfeit medicines have been almost overlooked or at least largely underestimated. It is now clearly established as one of the most crucial public health problems, mainly for the treatment of malaria in developing countries (Staehli *et al.*, 2013; Ingelman-Sundberg *et al.*, 2005; Relling, 1989). Counterfeiting of antimalarial drugs has received little attention at first compared to the considerable efforts made in other aspects of malaria control. Although important efforts are made to fight against this criminal traffic, much more needs to be done. Several very important and encouraging results have been obtained, but the problem will be completely solved if genuine antimalarials, free of charge are handed- over to population in subsahara African countries. Counterfeiting is more than a criminal act. Manslaughter is perfectly justified to describe such an act although some prefer calling it simply murder. It always involves unscrupulous people directing a highly organized technical and sophisticated criminal trade. These criminals make tablets out of starch, chalk, and a variety of wrong active ingredients for a life-threatening disease that particularly affects the poor populations who can least afford. These perpetrators knowingly manufacture these fake medicines and they fully know that their ineffective product may kill people who would otherwise survive their malaria infection. The illegal production, sale and distribution of fake drugs is a huge market evaluated to several billions of dollars and represents more than 50% of the pharmaceutical market in several African countries. Fake drugs have led to a very important number of deaths from untreated malaria or fatality provoked by toxic ingredients.

The overall findings suggested that there is no significant difference between pharmacists and public in the way they perceive counterfeit and substandard medicines. Both of them have the same susceptibility level toward counterfeit medicines. Despite the good quality of economic indicators in a country, both pharmacists and public have low-level of knowledge and moderate level of vulnerability to counterfeit and low-quality medicines.

Of the 15 batches analyzed, 6 (40%) were found to contain not enough active ingredients. This result could be link, considering low dosage, to therapeutic failures and resistance observed with treatments. This phenomenon had already been reported in Nigeria (Ehianeta *et al.*, 2012) with 50.8% under-dosed in artesunate and amodiaquine. These non-compliant samples all came from the public circuit of hospitals and health centers. This situation was also reported by Koumaré and collaborators (2016) who obtained 43 non-compliant samples, 40 of which were in hospitals and health centers. Samples used in our study were mainly imported from India with 33% of non-conformities, followed by Morocco (7%), these results are in the same line with those obtain by Koumaré (Koumaré *et al.*, 2016). In addition, we found that all the infant presentations collected were dosed with Amodiaquine. Indeed, Amodiaquine is transformed to a metabolite named "Amodiaquine desethyl" which is a prodrug, pharmacologically active and rapidly absorbed. Thus, low dosage and substandard Amodiaquine molecules could lead to therapeutic failures.

Globally, circulation of counterfeit drugs is responsible of deaths and cause damage to those who consume these medicines: toxic ingredients. However, these medicines are found within drug category financed by development aid. It is less clear how big this problem is relative to other problems that affect the physical and economic well-being of people in rich and poor countries. Unfortunately, the counterfeit market continues to grow and has considerably spread in the decade, especially in developing countries, often with the most dramatic consequences. This is the result of the wide-spread use of the internet to market counterfeit products in an unregulated environment of anonymity. Tackling this criminal traffic is the objective of an international program created by WHO and involves the international police and custom organizations like INTERPOL. International organization, IMPACT (International Medical Products Anti Counterfeiting Task Force) created by WHO in 2008, closely with INTERPOL and involving a total of 193 countries. In East Africa, a sub program of IMPACT, (operation MAMBA) (Ayala, 1978) led to the arrest of several manufacturers, wholesalers and retail pharmacists. Medicines for the treatment of malaria and antibiotics accounted for 64.5% of the samples, which reflects important public health concerns regarding antimicrobial resistance and drug-resistant infections. The aggregate observed failure rate of tested samples of substandard and falsified medicines in low and middle income countries is approximately 10.5%.

As Substandard and falsified medicines burden health systems by diverting resources to ineffective or harmful therapies, causing medical complications and prolonging diseases.They

reviewed 265 studies including 400,647 drug samples and meta-analysis of 96 studies with 67,839 drug samples, the prevalence of substandard and falsified medicines in low- and middle-income countries was 13.6% overall (19.1% for antimalarials and 12.4% for antibiotics).

Estimated economic impact data was mainly limited to market size and ranged widely from $10 billion to $200 billion. Significance Low-quality drugs and falsified medicines are an important health and economic problem; a concerted global effort is needed to secure the global supply chain, increase quality control capacity, and improve monitoring to better assess the problem and identify solutions. Nafiu and collaborators (2017) recommended the need to intensify such efforts in order to curb or totally eradicate the menace. At the end, they highlighted various strategies to curb the menace in order to stop its dangerous consequences for the public, at the national level, such elaboration of guidelines for National Drug distribution; Proper implementation of the Guideline; Establish a law on counterfeit and counterfeit medicines to ban the sale of drugs on the open market and usage of Mobile Authentication Services.

This trial showed that artemisinin-free SP+AQ was as effectiveas the artemisinin-based combination AS+AQ for the treatment of acute uncomplicated *P. falciparum* malaria in children aged 6 months to 10 years in the North and Far North Regions of Cameroon. SP+AQ showed a higher ACPR on day 28 without PCR correction (73.75%). However, after PCR correction AS+AQ had a slightly higher cure rate (91.8%) though the difference between the two drugs was not statistically significant (p=0.435). These results compete with similar studies in Mali (Kayentao *et al.,* 2009; Maiga *et al.,* 2015), where the highest ACPR was observed in the SP+AQ group. There were 24 (20.5%) ETF in the AS+AQ group and 21 (17.8%) in the SP+AQ group. After PCR correction there were 3 (2.6%) recrudescence rates in the AS+AQ group and 4 (3.4%) in the SP+AQ group. This figure was relatively low compared to a similar study conducted in the Democratic Republic of Congo (Swarthout *et al.,* 2006), which had relatively higher recrudescence rates in both AS+AQ and SP+AQ respectively. There was an increase in the hemoglobin levels from 9.96 ± 2.5 to 10.56 ± 2.4 in the SP+AQ group this was similar to studies in Mali (Kayentao *et al.,* 2009) and Cameroon (Basco *et al.,* 2002; Mbacham *et al.,* 2010), which showed a convincing increase in hemoglobin levels in children who were in the SP+AQ group, thus reducing anemia in this group. This increase was statistically significant (p=0.001). Therefore, SP+AQ is well tolerated in acute uncomplicated malaria and has no debilitating effects on patients with

anemia. There was also an increase in hemoglobin in the AS+AQ group, although this increase was not statistically significant.

As mentioned earlier, there were 7 cases recrudescence with 3 inthe AS+AQ group and 4 inthe SP+AQ group. With the analysis carried out to detect molecular markers of SP resistance, mutations in the *dhfr* gene were identified. Mutation at codon 59 was identified in all 7 recrudescent cases, hence revealing a C59R point mutation of 100% in these cases. Mutation at codon 108 was identified in 5 recrudescent cases with 2 in the AS+AQ group and 3 in the SP+AQ group. In total, 5 cases had double mutations for the *dhfr* gene (C59R+S108N). Studies in Cameroon (Mbacham *et al.,* 2010) and the Central African Republic (Nambei *et al.,* 2013), have shown that recrudescence or failure of treatment is associated with the presence of one or more molecular resistant markers, *dhfr* being one of these markers. Given that there was no analysis carried out on point mutations of the *dhfr* gene (codons 16, 51 and 164), there is a possibility that these recrudescence cases show triple or quadruple *dhfr* mutations. In addition, as the *dhps* gene was not analysed as well, it is also possible that dhfr / dhps haplotype mutations are described in the DRC (Swarthout *et al.,* 2006). Since resistance markers have only been analysed on the genome of the parasite, this may not have been the only cause of drug resistance in this population. It will be essential to explore the markers of host resistance and, in this case, the metabolic drug levels in the study participants will clearly show the association between drug resistance and gene mutations. In total, SP+AQ is as effectiveas AS+AQ in the treatment of acute uncomplicated malaria in children in this geo-ecological region of Cameroon.

One of the bottlenecks to effective treatment outcomes is polymorphisms in genes encoding enzymes responsible for drugs metabolism whereby individuals could either be slow, fast or intermediate metabolizers.

The CYP2C8 gene has several allelic forms known to be involved in drug metabolism. In this study, the CYP2C8*1 and CYP2C8*2 alleles were found, with allele CYP2C8*1 being predominant (76%) and alleles CYP2C8*2 less found (24%) in the study population (Figure 27). This result is in accordance with a study done in Senegal (15%) and Madagascar (15%) (Paganotti *et al.,* 2012), Tanzania (19%) (Marwa *et al.,* 2014), Burkina Fasso (15.5%) (Parikh *et al.,* 2007) and Ghana (17-17.5%) (Kudzi *et al.,* 2009). These alleles found in this study aided to distinguish the fast (CYP2C8*1/1), intermediate (CYP2C8*1/2) and slow (CYP2C8*2/2) metabolizers phenotypes, with fast metabolizer being predominant (70%) and

slow metabolizer phenotype less found (3.3%) (Figure 28), also in accordance with those observed in several countries, particularly in Senegal (55.7%), Uganda (79.7%), Madagascar (73.7%) and, 9%) (Paganotti *et al.,* 2012). According to these different phenotypes (Fast, Intermediate and Slow metabolizer) from the CPY2C8 gene, the risk to develop Early treatment failure (ETF) was determined and data demonstrated that the Intermediate metabolizers phenotype was at risk of developing ETF in this study population (table 20).

The NAT2 gene has several allelic forms known to be involved in drug metabolism. NAT2*4 (wild type) accounts for fast metabolizers, whereas NAT2*5, NAT2*6 and NAT2*7 are known to account for slow metabolisers. In this study, NAT2*4, NAT2*5, NAT2*6 and NAT2*7 alleles where found in the study population with NAT2*5 being predominant (39%) and alleles NAT2*7 less found (4%) in Garoua (Figure 33) consistent with findings in Egypt in 2010 and Jordan in 2003, whereby the NAT2*5 was also predominant, notably 50% among healthy Egyptians and 37.3% among unrelated Jordanian volunteers (Hamdy *et al.,* 2003; Jarrar *et al.,* 2010). The alleles found in our study helped to distinguish the different NAT2 (NAT2*4/4, NAT2*4/5, NAT2*4/6, NAT2*4/7, NAT2*5/5, NAT2*5/6, NAT2*5/7, NAT2*6/6 and NAT2*6/7) genotypes present in Garoua (figure 34). The most dominant genotype was NAT2*5/6 (28.12%) similar to results obtained in Bangolan, North West region of Cameroon but differed from the results obtained in Jordanian volunteers and in the North and South West regions of Cameroon whose main genotype was shown to be NAT2*5/6 (29.3%) and NAT2*4/6 (19%) respectively (Achonduh *et al.,* 2013; Jarrar *et al.,* 2010; Kengne *et al.,* 2016). These findings could be explained by the fact that, in as much as individuals are found in a given location, they could be different ethnic originsn (Sabbagh *et al.,* 2011). According to NAT2 phenotypes, intermediate metabolizers were dominant (61.4%) in Garoua. This results were similar to those obtained previously in Garoua, Mutengene and Bangolan (Achonduh *et al.,* 2013; Kengne *et al.,* 2016), where slow and intermediate metabolizers dominated. Furthermore, studies in the north, east and west of Nigeria as well as in Kenya assessing the polymorphism of the NAT2 gene had similar results. This is in concordance with findings showing that Africans are predominantly slow and intermediate metabolizers.

Inter-individual difference in drug metabolism rate is most likely due to human genetic polymorphisms (Belle *et al.,* 2008); based on this, the relationship between metabolism rates and treatment outcomes was determined. Data generated by the present study, demonstrate

that individuals with a fast metabolizer status were prone to experience early treatment failures (OR=4.792, P value=0.02), contrary to those with slow or intermediate metabolizer status. This relationship indicates that rapid metabolizers are more likely to have poor treatment outcomes. With regard to late parasitological failures, again, individuals with the fast metabolizer status were liable to have late parasitological failures (OR=4.333, P value= 0.047), contrary to those with slow and intermediate metabolizer status. This is in concordance with a 6 months' trial conducted in Japan, on tuberculosis patients using Isoniazid, in which patients with the fast metabolizer status were more susceptible to early treatment failures (Azuma *et al.,* 2013). While individuals with a fast metabolizer status are prone to poor treatment outcomes (early treatment failures and late parasitological failures), those with a slow and intermediate metaboliz status are much more susceptible to experiencing adverse effects to the prescribed drugs. This can be explained by the fact that fast acetylators metabolize xenobioticsis faster than the required time for therapeutic action to occur, hence, early treatment failures and late parasitological. Slow and intermediate acetylators on the other hand metabolize and eliminate xenobiotics slower than normal, exposing the human being to drug toxicity from drugs accumulation, and leading to adverse effects (Azuma *et al.*, 2013).

At the end, the cladogram, appropriate to conclude the synthetic analysis of the biomolecular result shows that slow metabolizers are more at risk of developing resistance mutation genes, different for rapid and intermediaites metabolizers. This is due to the fact that slow metabolizers delay their elimination of metabolites which probably increases the life of metabolites in the body; this status has contributed to improve the adaptation of the parasites and the development of resistance mutations.

CONCLUSION AND PERSPECTIVES

CONCLUSION

At the end of the study, the following conclusions emerged:

For the analysis of the situation of irrational use, the presence and extent of substandard/ falsified/ counterfeit antimalaria drugs in Cameroon, the conclusions are as follows:

Concerning the the impact of medicines quality on treatment failure,

- The majority of the non-compliant batches (**40%**), came from India,
- The low level of active ingredients found during the study, highlighted the urgent need to systematically monitor ACTs in Cameroon in order to prevent therapeutic failures and the risk of patient resistance to these combinations. The problem is quite serious especially since the children dosages were all represented in these nonconformities,
- It would also be advantageous to associate the therapeutic observance that is part of Continuous Pharmaceutical Care. Finally, quality control of all batches of ACT's as well as all other antimalarial drugs before their usage by the population within the Cameroonian territory should be the concern of the entire health system.

Concerning the pharmacoeconomic cost of the known presence of substandards/ falsified/ counterfeit antimalarials circulating in the official circuit,

- 40% of ASAQ tested were noncompliant to Artesunate and Amodiaquine Pharmacopeia standards.

For the investigation of the antimalaria efficacy of SPAQ versus ASAQ in the Northern Regions, with emphasis on clinical trials analysis and treatment outcomes, the conclusion is as follows:

- ETF and ACPR cases were recorded both in the AS+AQ and the SP+AQ groups on day 28. There were no observed cases of LCF nor LPF in both drug groups,
- There was no statistical significance in the difference in cure rates between the AS+AQ group and the SP+AQ,

- A very low recrudescence rate was observed in both study groups, with no case of reinfection and, the C59R mutation was observed in all recrudescence cases, while the S108N mutation was observed in 5 recrudescence cases.

For the determination of the pharmacogenomic profile of trial patients from Northern Regions of Cameroon by using CYP2C8 and NAT2 gene polyorphisms involved in the antimalaria drug metabolism, including their impact in treatment outcomes, the conclusions are as follows:

- The CYP2C8*1 and CYP2C8*2 alleles were found, with CYP2C8*1 being predominant (76%). According to the different phenotypes from the CPY2C8 gene, the Intermediate metabolizers phenotype was at risk of developing ETF (P=0.000).

- The NAT2*4, NAT2*5, NAT2*6 and NAT2*7 alleles of NAT2 gene were found in Garoua, NAT2*6 being the most predominant. The main genotype found was NAT2*5/6. According to the different phenotypes from the gene/protein, which interact directly with 10 (*NAT1, CYP1A1*, *CYP1A2, CYP2A6, CYP2D6, CYP2E1, GSTT1, GSTM1, XDH, C21 and f33*) others proteins, the fast phenotype was much more susceptible to experience early treatment failures (OR=4.792; p=0.02) and much more vulnerable to experience late parasitological failures (OR=4.333, p=0.047).

- The construction of the cladogram helps to identify three phenotypes: Cluster A with patients who are intermediate metabolizers, without pfDHFR S108N and pfDHPS C58R, resistance markers; Cluster C with rapid metabolizers, without the 02 resistance markers; Cluster B with slow metabolizers and with the 2 resistancemarkers.

PERSPECTIVES AND RECOMMENDATIONS

1. Pespectives

To Researchers

- To carry out an extensive study with a larger sample size to master the real state of irrational use, presence and extent of substandard/ falsified/ counterfeit antimalaria drugs in Cameroon,
- To carry out an extensive molecular epidemiology study with a larger sample size on the genes of resistance to the used antimalarials should be carried out in all geo-ecological zones of Cameroon to understand the evolution of mutations in the known genes of resistance in the country,
- Emphasis on the human genetic profiles based on more candidate gene polymorphisms of ASAQ metabolising enzymes should be made in subsequent trials to better understand the cause of recrudescence or re-infection in treatment failures.

2. Recommendations

To the Cameroon Government

- To provide the resources to promote research in this domain as well as synergy between academia and the ministry of Public Health.

To Policy Makers

- To ensure that counterfeit medical produc+ts is a crime and that punishment is commensurate to the consequences that it has on personal health and on the credibility of national health care delivery systems,
- To ensure that all manufacturers, importers, exporters, distributors and retailers comply with the appropriate requirements that are necessary for a secure distribution chain for all medical products,

To Health Personnel

- To ensure that health professional, the general public and the medias are aware of the dangers associated with counterfeit medicines.
- Educate the population on correct behavior and choices.

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APPENDIX

Appendix 1 : Ethical Clearance

COMITE NATIONAL D'ETHIQUE DE LA RECHERCHE POUR LA SANTE HUMAINE

Arrêté N° 0977/A/MINSANTE/SESP/SG/DROS/ du 18 avril 2012 portant création, organisation et fonctionnement des comités d'éthique de la recherche pour la santé humaine au sein des structures relevant du Ministère en charge de la santé publique

N° 2015/03/567/CE/CNERSH/SP Yaoundé, le 13 mars 2015

Cnethique_minsante@yahoo.fr

CLAIRANCE ETHIQUE

Le Comité National d'Ethique de la Recherche pour la Santé Humaine (CNERSH), en sa session ordinaire du 31 octobre 2014, a examiné le projet de recherche intitulé : **«Antimalarial Combinations and Clinical Response in North and Far North Cameroon: Artesunate + Amodiaquine (ASAQ) Vs Sulphadoxine-pyrimethamine + Amodiaquine (SPAQ)»,** soumis par le **Professeur MBACHAM Wilfred**, Investigateur Principal, Université de Yaoundé1.

Le projet est d'un grand intérêt scientifique et social. La procédure de l'étude est bien documentée et claire. Les risques liés à l'étude sont présentés ainsi que les mesures pour les éviter et les minimiser. La notice d'information et le formulaire de consentement éclairé, en français et en anglais, sont bien élaborés et simples à comprendre. Les mesures prises pour garantir la confidentialité des données collectées sont présentes dans le document. Les CVs des Investigateurs les décrivent comme des personnes compétentes, capables de mener à bien cette étude. Pour toutes ces raisons, le Comité National d'Ethique approuve pour une durée d'un an, la mise en œuvre de la présente version du protocole.

Les Investigateurs sont responsables du respect scrupuleux du protocole approuvé et ne devraient y apporter aucun amendement aussi mineur soit-il, sans avis favorable du CNERSH. Les investigateurs sont appelés à collaborer pour toute descente du CNERSH pour le suivi de la mise en œuvre du protocole approuvé. Le rapport final du projet devra être soumis au CNERSH et aux autorités sanitaires du Cameroun.

La présente clairance peut être retirée en cas de non respect de la réglementation en vigueur et des recommandations susmentionnées.

En foi de quoi, la présente clairance éthique est délivrée pour servir et valoir ce que de droit.

Ampliations

- MINSANTE

Le Président

Pr Lazare KAPTUE

N.B : cette clairance éthique ne vous dispense pas de l'autorisation administrative de recherche (AAR), exigée pour mener cette étude sur le territoire camerounais. Cette dernière vous sera délivrée par le Ministère de la Santé Publique.

Appendix 2 : Informed Assent Form

The Biotechnology Centre, Box 8094 Yaoundé
University of Yaoundé, Cameroon

Project Title: **Research on Anti-Malaria Drug Efficacy in Cameroon: Safety and Efficacy of Sulfadoxine +Pyrimethamine and Amodiaquine versus Artesunate Amodiaquine in North and Far North Cameroon.**

Principal Investigator: **Pr. Wilfred F. Mbacham**

Study Number _____

1. *Purpose*

There are many drugs that can be used to treat malaria. These old drugs no longer cure well. Many other drugs are being sold. Some others are being newly made and will be sold soon. Some of these new drugs are a combination of more than one antimalarial drug. In this study, we are trying to compare how well the new drugs such as Artesunate-Amodiaquine (Co-Arsucam®), and Sulfadoxine/Pyrimethamine-Amodiaquine are effective in the treatment of malaria.

Your child is invited to participate in this study because your child is suffering from malarial infection. We will ask the permission of parents of 250 children aged 6 months to 10 years with acute malarial infection to take part in this study.

2. *Procedure*

If you agree for your child to be in the trial, you will be asked some questions about how you have treated your child in the past. Your address will be requested so that we can visit your sick child. A study doctor or nurse will examine your child. We will prick your child's finger to take a few drops of blood to look for parasites. We will also take blood from the vein of your child, the volume of the tip of a teaspoon, to do some laboratory tests and to find out why some people respond quickly when treated and other people do not. Your child will be assigned into one of two groups to receive either Artesunate-Amodiaquine or Sulfadoxine/Pyrimethamine-Amodiaquine. The assignment will be decided by chance. You and the study staff will not know which group your child will be in. Your child will be given the drug at the hospital every day for 3 days. We will ask you to return with your child to the study clinic on days 3, 7, 14, 28, or at any other time if your child feels unwell. If you and your child do not come to the clinic, we will visit you at your home. On follow-up days (including the day when clinical sign/symptom of malaria appears), your child will be examined and assessed for clinical symptoms of malaria. We will prick your child's finger to take blood to look for the presence of malaria in the blood and its genetics (how the parasite is made up inside). At some scheduled visits, we will also take blood from your child to do some tests such as blood cell count and sugar level.

3. Risks and discomforts

Most children will have no problems with the drugs given but occasionally they may develop mild itching, rashes or intestinal upsets, headache or blurred vision.

4. Benefits

The drugs may help your child. However, this cannot be guaranteed. Allowing your child to take part in this study will benefit the community by helping to tell the doctors which drugs are good and how to use the new drugs for malaria.

5. Cost

All malaria and additional tests, drugs and hospital fee for staying in hospital during your child's involvement in the study are for free.

6. Transport

We will pay for your transport to bring your child back to the clinic**.**

7. Alternatives

If you decide not to take part in the study, your child will still receive the standard care in this hospital.

8. Confidentiality

The clinical and laboratory data recorded will be kept confidential and used for this research only. The results of this research may be published in scientific journals or presented at medical meetings, but your identity will not be disclosed.

9. Injury and Compensation

If your child has experienced any research-related illness or injury, you can contact the medical doctor at this clinic who will pay any charges required for the treatment of study related illness/injury.

10. New information and result of the study

It is possible that new information becomes available about the medicines used in this study. If this happens, the study doctor will tell you about it and also discuss with you whether you want your child to continue in the study. If you decide not to continue, the study will still arrange for your child's care to continue. If you decide to continue in the study you will be asked to sign an updated parental permission form. The investigator will also inform you about the progress and outcome of the research.

11. Questions

If you feel you have not been properly told as to the discomfort, benefits or your rights, please feel free to take the matter to your local head of the hospital or contact the following person: *Prof Marie Claire Okomo, National Ethics Committee, Centre Region Cameroon*. Telephone: (+237) 6*99767427.*

12.Voluntary Participation

Your child's participation in this study is purely voluntary and you (your child) will be given sufficient time to decide whether or not to take part. You (and your child) may refuse to participate at any time

and still benefit from full treatment for malaria. There will be no injustice, punishment, loss of benefits to which you (or your child) are otherwise entitled at this hospital.

13. Acceptance

My questions concerning this study have been answered by ----------. Or I have read the information sheet concerning this study [or have understood the verbal explanation] and I understand what will be required of me and what will happen to my child if I take part in it. I understand that at any time I may withdraw my child from this study without giving a reason and without affecting normal care and management. **I agree that my child should take part in the study.**

Name of Interpreter _____ Date _____

Child's name _____

Parent's/guardian's signature (or thumb print): _____Date_____

Parent's/guardian's Printed Name*:* _____

Witness's Signature: _____ Date: _____

Witness's Printed Name: _____

Researcher Recognizance

The subject has been fully informed of the nature and purpose of the procedures described above including any discomfort involved in its performance. The subject has been asked if any questions have arisen regarding the procedures and these questions have been answered to the best of the investigator's ability.

Signature of Investigator:

Appendix 3 : Doses of study drugs

A. Study Arm: Artesunate-Amodiaquine

Drug	Presentation	Age	Day 0	Day 1	Day 2
Artesunate-Amodiaquine	25mg/67,5mg	6-11 months	1 tablet	1 tablet	1 tablet
		1-5 years	2 tablets	2 tablets	2 tablets
	100mg/270mg	6-10 years	1 tablet	1 tablet	1 tablet

B. Study Arm: Sulfadoxine/Pyrimethamine-Amodiaquine

Drug	Presentation	Age	Day 0	Day 1	Day 2
Sulphadoxine-Pyrimethamine	500mg/25mg	6-11 months	½ tablet	N/A	N/A
		1-5 years	1 tablet	N/A	N/A
		6-10 years	1½ tablets	N/A	N/A
Amodiaquine	153mg	6-11 months	½ tablet	½ tablet	½ tablet
		1-5 years	1 tablet	1 tablet	1 tablet
		6-10 years	1½ tablets	1½ tablets	1½ tablets

Appendix 4 : Case Report Form

PARTICIPANT INFORMATION SHEET

TITLE: "DISTINGUISHING BETWEEN SLOW AND FAST METABOLISERS OF XENOBIOTICS FOLLOWING A MALARIA CLINICAL TRIAL."

Co-INVESTIGATOR: Mbekuveh DINGA-NYOH

INVITATION: This is an invitation to participate in a study that aims at distinguishing between slow and fast metabolisers of xenobiotics following a malaria clinical trial and to decipher their individual relationships with treatment outcomes.

AIM OF THE STUDY:

General Objectives

To determine the role of pharmacogenomic factors in treatment outcomes among individuals with uncomplicated malaria in Garoua.

Specific Objectives

- To determine the main SNPs in NAT2 gene.
- To find the association between SNPs (single nucleotide polymorphisms) in NAT2 genes and anti-malarial treatment outcomes.
- To identify genetic/proteinic factors which alongside NAT2 have an effect on treatment outcomes.

DURATION OF STUDY: From November 2016 to May 2017

STUDY POPULATION: All patientsbetween 6months to 10 years with uncomplicated malaria.

PROCEDURE: The patients, after agreeing to participate in the study, will sign a consent form. They shall then be examined clinically and blood samples will be taken thereafter, all with the aim of assessing for eligibility. This will last for approximately 1hour 30minutes. Eligible patients' blood samples will be further used for laboratory investigations (PCR). The data collected will be analysed using appropriate statistical methods and the results obtained may eventually be published in a scientific document. You will have access to your results at the end of the study.

SAMPLED BLOOD: The sampled blood will be analysed in a recommended laboratory disposed after analysis.

PARTICIPATION: The participant is free to accept or deny the proposal, to withdraw at any time in the course of the study.

ETHICAL ISSUES: Ethical clearance will be obtained from the Institutional Ethical Review Board of the Faculty of Medicine and Biomedical Sciences of the University of Yaoundé I. All the biological tests performed will be free of charge. The duration of study, repeated finger pricks could result in pain, bleeding and infection. The total amount of blood collected will be about 50-200microlitres per encounter. If adverse effects were to occur, participants will be withdrawn from the study and managed with a rescue treatment. Their results shall be kept confidential and private. Denial to participate will not affect your follow up in the service. For more information or further clarifications about the study you can contact this Co-investigator Mbekuveh DINGA-NYOH, BP 8046 Yaoundé, Cameroon through the following telephone number: (+237) 673 93 9182 and e-mail address: vehnyoh@yahoo.com

FICHE D'INFORMATION DU PARTICIPANT

TITRE: " DISTINCTION ENTRE LES METABOLISEURS LENTS ET RAPIDES DES XENOBIOTIQUES SUITE A UN ESSAI CLINIQUE DE PALUDISME"

Co-INVSTGATRICE : Mbekuveh DINGA-NYOH

APPEL A PARTICIPER : Ceci est une invitation à participer à une étude avec pour objectif, de distinguer entre les metabolizeurs lents et rapides des xenobiotiques suite à un essai clinique de paludisme et investiguer leur relation avec l`issue de traitement.

OBJECTIFS DE L'ETUDE :

Objectifs Généraux

L'étude vise à enquêter le rôle des facteurs pharmaco génomique dans l'issue du traitement chez les individus atteint du paludisme non-complique à Garoua.

Objectifs Spécifiques

Identifier la présence des PSNs sur le gène NAT2.

Enquêter sur l'association entre les PSNs sur le gène NAT2 et l'issue de traitement antipaludéen.

Décrire la relation entre le gène NAT2 et d'autre gènes métabolisant des antipaludéens.

DUREE DE LETUDE : De Novembre 2016 à Mai 2017

POPULATION SOUS ETUDE : Tout patient présentant des symptômes de paludisme simple.

PROCEDURE : les malades, après acceptation de participer à cette étude, seront invités à signer un formulaire de consentement. Ils vont par la suite subir une examination clinique et une prise de sang effectuée avec pour objectif, l'évaluation de leur éligibilité pour l'étude. Ceci durera sensiblement 1 heure 30 minutes par malade. Les malades seront traitées avec artesunate-amodiaquine a des doses recommander. Le sang des malades qui s'avéreront éligible fera l'objet plus tard des tests en laboratoire (PCR). Les données obtenues seront analysées et traitées utilisant des méthodes statistiques indiquées et les conclusions seront publiées dans une revue scientifique. Le malade aura aussi droit aux résultats individuels révélés par les tests en laboratoire, à la fin de l'étude.

SANG ECHANTILLONNE : Le sang échantillonné sera analysé dans un laboratoire recommandé et jeté après analyse. .

CONDITIONS DE PARTICIPATION : Tout participant ayant donné son consentement joui de son droit d'y renoncer et signaler sa volonté à ne plus participer sans toutefois subir le moindre préjudice au niveau de sa prise en charge normale dans l'unité de traitement.

QUESTIONS ETHIQUES : L'approbation éthique pour l'étude sera octroyée par la Commission d'éthique Institutionnel de la faculté de Médecine et sciences Biomédicales de l'Université de Yaoundé I. Tous les tests biologiques seront entièrement gratuits. Durant l'étude, les piqures répétées des doigts peuvent engendrer la douleur, saignement et possible infection. L'échantillonnage sera faites par l'enquêteur principal assisté de personnel médicaux qualifiés. Le taux de sang par prise sera compris entre 50-200 microlitres. Au cas où un patient montrera des signes d'effets adverses il/elle sera immédiatement retiré de l'étude et suivi par un traitement de secours. Après consentement à participer chaque participant se verra affecte un code afin que leurs noms ne figurent pas ni dans un document de recherche ni diffusé dans la communauté de recherche. En plus de cela, leurs résultats issus de laboratoire seront aussi confidentiels et privé.

Pour toute autre information et clarifications par rapport à l'étude veuillez entrer en contact avec cette Co-Investigatrice Mbekuveh DINGA-NYOH, BP 8046 Yaoundé, Cameroun. Elle répond aux cordonnés suivants : téléphone; (+237) 673 93 9182 e-mail: vehnyoh@yahoo.com

Appendix 5 : NIH Common Toxicity Criteria

FIRST PAGE

Adverse Event	Grade 0	1	2	3	4
ALLERGY/IMMUNOLOGY					
Allergic reaction/ hypersensitivity (including drug fever)	none	transient rash, drug fever <38°C (<100.4°F)	urticaria, drug fever ≥38°C (≥100.4°F), and/or asymptomatic bronchospasm	symptomatic bronchospasm, requiring parenteral medication(s), with or without urticaria; allergy-related edema/angio edema	Anaphylaxis
Note: Isolated urticaria, in the absence of other manifestations of an allergic or hypersensitivity reaction, is graded in the DERMATOLOGY/SKIN category.					
Allergic rhinitis (including sneezing, nasal stuffiness, postnasal drip)	none	mild, not requiring treatment	moderate, requiring treatment	-	-
Autoimmune reaction	none	serologic or other evidence of autoimmune reaction but patient is asymptomatic (e.g., vitiligo), all organ function is normal and no treatment is required	evidence of autoimmune reaction involving a non-essential organ or function (e.g., hypothyroidism), requiring treatment other than immunosuppressive drugs	reversible autoimmune reaction involving function of a major organ or other adverse event (e.g., transient colitis or anemia), requiring short-term immunosuppressive treatment	autoimmune reaction causing major grade 4 organ dysfunction; progressive and irreversible reaction; long-term administration of high-dose immuno-suppressive therapy required
Also consider Hypothyroidism, Colitis, Hemoglobin, Hemolysis.					
Serum sickness	none	-	-	present	-

LAST PAGE

Masculinization of female is graded in the ENDOCRINE category.					
Vaginal dryness	Normal	mild	requiring treatment and/or interfering with sexual function, dyspareunia	-	-
Sexual/Reproductive Function - Other (Specify, _____)	None	mild	moderate	severe	Disabling
SYNDROMES (not included in previous categories)					
Acute vascular leak syndrome is graded in the CARDIOVASCULAR (GENERAL) category.					
ARDS (Adult Respiratory Distress Syndrome) is graded in the PULMONARY category.					
Autoimmune reactions are graded in the ALLERGY/IMMUNOLOGY category.					
DIC (disseminated intravascular coagulation) is graded in the COAGULATION category.					
Fanconi's syndrome is graded as Urinary electrolyte wasting in the RENAL/GENITOURINARY category.					
Renal tubular acidosis is graded as Urinary electrolyte wasting in the RENAL/GENITOURINARY category.					
Stevens-Johnson syndrome (erythema multiforme) is graded in the DERMATOLOGY/SKIN category.					
SIADH (syndrome of inappropriate antidiuretic hormone) is graded in the ENDOCRINE category.					
Thrombotic microangiopathy (e.g., thrombotic thrombocytopenic purpura/TTP or hemolytic uremic syndrome/HUS) is graded in the COAGULATION category.					
Tumor flare	None	mild pain not interfering with function	moderate pain; pain or analgesics interfering with function, but not interfering with activities of daily living	severe pain; pain or analgesics interfering with function and interfering with activities of daily living	Disabling
Also consider Hypercalcemia Note: Tumor flare is characterized by a constellation of symptoms and signs in direct relation to initiation of therapy (e.g., anti-estrogens/androgens or additional hormones). The symptoms/signs include tumor pain, inflammation of visible tumor, hypercalcemia, diffuse bone pain, and other electrolyte disturbances.					
Tumor lysis syndrome	Absent	-	-	present	-
Also consider Hyperkalemia, Creatinine.					
Urinary electrolyte wasting (e.g., Fanconi's syndrome, renal tubular acidosis) is graded in the RENAL/GENITOURINARY category.					
Syndromes - Other (Specify, _____)	None	mild	moderate	severe	life-threatening or disabling

Appendix 6 : Preparation of solutions

EDTA 0.5M (500ml)

Weigh 93,1g of EDTA (ethylene diamine tetra acetic acid) then add 400ml of distilled water. Add concentrated NaOH to obtain a pH of 8 at which EDTA dissolves. Fill up to 500ml with distilled water and store at ambient temperature.

Chelex-100 à 20% (BIORAD)

Weigh 20g de chelex-100 (powder) and add 50ml of sterilized Phosphate Buffer Solution (PBS). Mix, then let suspension settle. Dispose supernatant then add PBS to 100ml. Store at 4°C.

2% Agarose Gel

Weigh 1g of agarose (powder) then add 50ml of TBE 1X. boil and leave to cool. Add 2.5µl of ethidium bromide and mix gently. Poor into the gel mould and allow to solidify.

Ethidium Bromide (10ml) (EtBr)

Weigh 0,15g of EtBr and dissolve in 10ml of doubly distilled water. Conserve at room temperature in an opaque tube sealed with aluminium foil paper.

Saponine 0, 5%

Weigh 0,25g of saponine and add 50ml of PBS 1X, then sterilize in autoclave and store at 4°C for 48hours.

Buffer-TE (100ml)

Aliquot 1ml of Tris-HCl 1M/pH 8 and add 200µl of EDTA 0,5M. Fill up to 100ml with distilled water. Sterilize and store at room temperature.

10X PBS pH 7.2 (500ml)

Weigh 77.75mg of Na_2HPO_4, 10.2g de NaH_2PO_4 and 190.84g of NaCl. All are dissolved in 300ml of distilled water and the pH adjusted to 7.2 with HCl 1M. Fill up to 500ml using distilled water. Store at room temperature.

Tris-HCl 1M pH 8 (1Litre)

Weigh 121,1g of Tris and add 800ml of distilled water. Use a concentrated solution of HCL to adjust pH to 8. Fill up to 1litre using distilled water. Sterilize and store at 4°C.

10xTBE (1Litre)

Weigh 5.4 g of boric acid, 108g of Tris base and aliquot 40ml of 0.5M EDTA pH8. Shake vigorously, then fill up to 1litre with distilled water.

Molecular weight marker (200µl)

Pipette 140µl of buffer-TE 1X, sterilize, then add 20µl of DNA marker. Fill up to 200µl using the loading buffer. Store at -20°C.

Tampon d'échantillon pour electrophorese

Weigh 0.125g of bromophénol blue 0.25% ; 0,125g of xylène cyanol 0,25% ; 7,5g of Ficoll 15%. Mix all in a sterile tube and add 2ml of EDTA 0,5M. Fill up to 50ml using distilled water. Mix and store at room temperature.

HCl 5M (100ml)

Pipette 41.5ml of stock HCl (37%, densité 1.19). Fill up to 100ml using distilled water. Store at room temperature.

dNTPs 10mM

Pipette 20µl of each dNTP (dATP, dTTP, dCTP, dGTP). Fill up to 200µl using sterilised bufferTE 1X autoclavé. Store at -20°C

Appendix 7 : Consent Form

Mr. / Mrs. / Ms.....

Title: “DISTINGUISHING BETWEEN SLOW AND FAST METABOLISERS OF XENOBIOTICS FOLLOWING A MALARIA CLINICAL TRIAL”.

The co-Investigator, Mbekuveh DINGA-NYOH. The principal investigator is Pr. MBACHAM Wilfred. The study seeks investigate the role of pharmaco-genomic factors in treatment outcomes among individuals suffering from uncomplicated malaria in Garoua.

She laid emphasis on the fact that I was free to accept or deny to let my child participate in the study. I have received and understood the following information:

- The aim of this study, the procedure and the tests to be done
- Possible constraints and risks

I accept that my medical history be consulted by the research personnel and used for research purposes only. My medical record will be discussed with me at the end of the study. My participation can be interrupted at any time if the principal investigator deems it necessary or if I wish. All data concerning me will be strictly confidential. Only the research personnel, and eventually a health authority representative will be given access to my data. The research protocol for this study has been reviewed and validated by the National Research Ethical Committee. At any time, I can ask for supplementary information from the co-investigator, Mbekuveh DINGA-NYOH, using the phone number: 673-93-91-82.

I hereby accept to participate in the study under the aforementioned conditions. A signed copy of this consent form will be given to me and will serve its purpose in time of need.

Date: ...../...../.....

Investigator’s signature Volunteer’s signature

Appendix 8 : Molecular Data Summary

Sample ID	C58R	S108N	Double mutation	Drug
GRA 3002	1	1	1	AQSP
GRA 3010	1	1	1	AQSP
GRA 4004	1	1	1	ASAQ
GRA 4010	1	1	1	AQSP
GRA 6004	1	1	1	ASAQ
YGA 5014	1	0	0	AQSP
YGA 5021	1	0	0	ASAQ

Appendix 9 : Genetic distances based on DHFR, DHPS, AND NAT2 GENES

	G101	G110	G111	G112	G113	G114	G115	G116	G117	G118	G102	G103	G104	G105	G106	G107	G108	G109	G21	G210	G211	G212	G213	G214	Y115	Y116	Y217	Y218	G219	Y202
G101																														
G110	1																													
G111	0	1																												
G112	0	1	0																											
G113	0	1	0	0																										
G114	0	1	0	0	0																									
G115	1	0	1	1	1	1																								
G116	0	1	0	0	0	0	1																							
G117	1	0	1	1	1	1	0	1																						
G118	0	1	0	0	0	0	1	0	1																					
G102	1	0	1	1	1	1	0	1	0	1																				

G103	0,5	1	0,5	0,5	0,5	0,5	1	0,5	1	0,5	1																			
G104	1	1	1	1	1	1	1	1	1	1	1	1																		
G105	0	1	0	0	0	0	1	0	1	0	1	0,5	1																	
G106	0	1	0	0	0	0	1	0	1	0	1	0,5	1	0																
G107	0	1	0	0	0	0	1	0	1	0	1	0,5	1	0	0															
G108	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1														
G109	1	0	1	1	1	1	0	1	0	1	0	0,75	1	1	1	1	1													
G21	0	1	0	0	0	0	1	0	1	0	1	0,5	1	0	0	0	1	1												
G210	0	1	0	0	0	0	1	0	1	0	1	0,5	1	0	0	0	1	1	0											
G211	0	1	0	0	0	0	1	0	1	0	1	0,5	1	0	0	0	1	1	0	0										
G212	1	0,33	1	1	1	1	0,33	1	0,33	1	0,33	0,66	1	1	1	1	1	0,33	1	1	1									
G213	1	0	1	1	1	1	0	1	0	1	0	0,75	1	1	1	1	1	0	1	1	1	0,33								
G214	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1							

p

Y115	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0							
Y116	0	1	0	0	0	0	1	0	1	0	1	0,5	1	0	0	0	1	1	0	0	0	1	1	1	1						
Y217	0	1	0	0	0	0	1	0	1	0	1	0,5	1	0	0	0	1	1	0	0	0	1	1	1	1	0					
Y218	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	0	1	1				
G219	0,5	1	0,5	0,5	0,5	0,5	1	0,5	1	0,5	1	0	1	0,5	0,5	0,5	1	1	0,5	0,5	0,5	0,66	1	1	1	0,5	0,5	1			
Y202	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	1	0	1	1	1	1	1	1	0	0	1	1	0	1		

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Appendix 10 : List of Publications

Rose Ngono Mballa, Jean Paul Chedjou, Randolph Ngwafor, EkolloAristid, Innocent Ali, AkindehNji, Forsah-Achu Dorothy, Ekoyol Ekobe Germaine, Olivia Achondu, Dinga-Nyoh Mbeku Veh, Wilfred Fon Mbacham. (2019). **Single nucleotide polymorphisms in the *cyp2C8* and *nat2* genes and treatment outcomes in patients suffering from uncomplicated malaria in Garoua, Northern Region of Cameroon.** *Pharmacy & Pharmacology International Journal*; 7(4):147–153