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ANTI-MALARIAL DRUG RESISTANCE IN CAMEROON

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Report of a Workshop Held at Hilton Hotel Yaoundé, Cameroon January 31st – February 1st, 2013

Published by

The Cameroon Academy of Sciences
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NOTICE

The project that is the subject of this study was supported by grant agreement n°IOM-5855-05-002 between the United States National Academy of Sciences and the Cameroon Academy of Sciences.

International Standard Book Number **9956-26-38-x**

Additional copies of this publication are available from the Cameroon Academy of Sciences, P.O. Box 1457 Yaoundé, Cameroon or http:www.casciences.com

Cover designed by Professor Wilfred Mbacham.

Citation: CAS (2014). Anti-malarial drug resistance in Cameroon. Workshop Report. Cameroon Academy of Sciences, Yaoundé, Cameroon.

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Acknowledgements

In general, the Cameroon Academy of Sciences gratefully acknowledges the organisations and individuals whose contributions and efforts made the organisation of this workshop possible.

Specifically, we are indebted to Dr. Madeleine Tchuinte, the Minister of Scientific Research and Innovation (MINRESI) for her continuous support to CAS. We thank the USNAS for providing financial and technical support for the workshop and the publication of this report. Our special thanks go to Dr. Patrick Kelly, Ms Patricia Cuff, Mr Jim Banihashemi, Mr Christian Acemah and Ms Angela Christian for facilitating the collaboration between CAS and the USNAS.

We express our gratitudeto the Chair (Prof. Wali Muna) and members of the CAS Forum on Public Health for programming the workshops on public health.

We owe immense gratitude to the Fobang Foundation for assigning MrNguetti Honore to CAS as a volunteer for 6 months and for accepting the use of sections of the Cameroon Research and Control Report 2008 -2011 that was published in 2012.

We express our sincere thanks to the chair (Prof. Wilfred Mbacham) and members (Prof. W. Muna, Prof. Rose Leke, Prof. Roger Somo Moyou) of the local organizing committee, advocacy partners such as MC-CCAM, MnM and Clinton Foundation (CHAI) and the secretariat team (Drs. David Mbah, Vincent N. Tanya, Jude Bigoga and Palmer Netongo and Messrs Ngeutti Honore and Ego Thaddeus). We also thank the session moderators and rapporteurs who took down notes that made this report possible. These include Prof. V.P.K. Titanji, Prof. Rose Leke, Dr. Tourgordi Alexis, Dr. Jean Bickii, Dr. Awono Ambene, Mr Chedjou Kengne Jean Paul and Mr Ekollo Mbange Aristide.

We appreciate Prof. Mbacham for writing this report. The review and editing process for the report was overseen by Dr. Vincent N. Tanya, Programme Officer of CAS.

Foreword

A series of meetings were held at the Cameroon Academy of Sciences office to fine tune the agenda for the workshop. Further consultations were held with Dr. Patrick Kelly and Mr Acemah Christian of United States National Academy of Sciences (USNAS).Given the above, it was then resolved that a two day meeting will be organized under the auspices of the CAS National Public Health Forum on "Anti-Malarial Drug Resistance in Cameroon" with topics to include: Quality of drugs, emergence of drug resistance and evolution of drug policies and measures to contain these. The meeting was aimed at:

1. Rallying experts around the table,
2. Debating the state of anti-malaria drug resistance in Cameroon and Africa,
3. Examining the practices that may generate these resistances,
4. Examining the systems issues that may be involved in appropriate treatment, and
5. Addressing new approaches towards containment of resistance.

The targeted audience included policy makers (from the ministries of health, scientific research and innovation and higher education), advocacy groups, international NGOs and Academia. Other stakeholders invited included embassies (France, Brazil, USA, China, UK, and German) and donors (the World Bank and the African Development Bank). To steer the logistics of the workshop, a local organizing committee was set up with Prof. Wilfred Mbacham as its chair. The members included Prof. W. Muna, Prof. Rose Leke, Prof. Roger Somo Moyou and advocacy partners such as MC-CCAM, MnM and Clinton Foundation (CHAI). The secretariat was composed of Drs. David Mbah, Vincent Tanya, Jude Bigoga and Palmer Netongo and Messrs Ngeutti Honore and Ego Thaddeus.

Prof. Wali Muna
Chair National Public Health Forum

Executive Summary

The Cameroon Academy of Sciences convened about 60 researchers from Universities and Research Institutes from 31st January to 1st February 2013 at the Yaoundé Hilton Hotel to examine the malaria drug resistance situation in Cameroon. The workshop was structured into blocks of 4-5 talks followed by discussions.

In the debate that ensured, it was observed that overuse of Artemisinine-based Combination Therapies (ACTs) in Cameroon could lead to resistance especially if not accompanied by adequate education to match shifting guidelines. Since 2002, the policy changed from monotherapies to ACTs in 2004. Artesunate-Amodiaquine (AQ) was the first-line and Arthemeter-Lumefantrine was used for second line treatments. In 2006, for severe malaria, quinine was adopted as the alternative in wait of injectable Arthemeter. A national consensus was arrived at to include RDTs for diagnosis in 2009 as part of the treatment algorithm. The ASAQ, AL and dihydro-artemisinine – piperaquine (DHAP) introduced into the Cameroon markets were shown to be of comparable efficacy. This is also the case for *in vivo* efficacy of Quinine sulfate (QS) for uncomplicated malaria assessed and demonstrated to be at 94.2% in the South West Region of Cameroon. The overuse of ACTs and quinine within the private sector that can occur especially when malaria treatment is provided to patients who do not have malaria (parasite negative patients) may lead to the selection of resistance parasites. *It is therefore necessary that treatment must be based on the parasitic demonstration of Plasmodium spp through the use of Rapid Diagnostic Tests (RDTs) or microscopy. Training schools in the field of health need to be brought up to date on these guidelines.*

Mutations on drug metabolism enzymes as well as microsatellite markers that flank these genes stand as good markers in forensic science, genetic diversity and resistance patterns establishment. These were used to demonstrate a clear picture on resistant lineages for Cameroon, with the North differing from the south on the basis of markers of resistance on *dhps, dhfr, pfmdr1* and *pfcrt* genes. Resistant lineages of *dhps* from Cameroon when placed in the context of other countries in Africa, clustered alone as one of 5 other variant, with the conclusion that resistant lineages might have occurred and spread at different time in Africa. With the emergence of resistance to Artemisinines already reported in South East Asia, *it is required of us to optimize current drug use and develop new formulations towards malaria eradication*. These may mean that we go back to the drawing board to redefine mechanisms of resistance, pharmacological strategies, and immunological presentations in vulnerable populations. *We need affordable technologies that allow ultrasensitive same-day, on-site diagnostics in hundreds of patients. Pursuing the policy of presumptive therapies may be temporarily cost-effective but may ultimately lead to long-term increase in parasite resistance.* Acute *P. falciparum* malaria might be treated with 12 or more therapies and half as many are in clinical development. However, the only treatment for the asexual liver stages and the sexual blood stages of all species is primaquine and the addition of one dose of primaquine to artemisinine-based combination regimens could help to counter the spread of artemisinine resistance. The complexity of the malaria parasite itself and its lifecycle mean that designing a vaccine is more problematic than for, say, viruses. And some researchers feel that a single recombinant protein subunit is unlikely to ever provide adequate protection to be of general use.

In Public Health approaches to malaria prevention, the NMCP was noted to proceed in two ways - vector control through use of LLIN and IRS and chemoprevention. With difficult diagnosis and inflammatory reactions in the placenta, malaria during pregnancy has deleterious consequences for

the mother and the foetus. IPTp works and greatly reduces peripheral and placental parasitaemia. However, published studies demonstrate that IPTp coverage for the treatment of malaria in pregnancy needs to be stepped up from the current very low 60%. Mass drug therapy or chemoprevention is an option to prevent inter-season transmission since these therapies are regarded as a public health response to an ever surging pandemic. *Priority areas of focus include the rational use of drugs in vulnerable populations and the understanding of pharmacokinetics and pharmacodynamics of the current practice of intake of herbal formulations in combination with formulated drugs*.

The loss of ACTs as an efficacious malaria treatment would severely jeopardize efforts to control and ultimately eliminate malaria in Sub-Saharan Africa. The spread of this resistant parasite to Sub-Saharan Africa would represent a public health catastrophe. *Therefore rational use of ACTs only in patients with confirmed malaria is critical*. Treatment and prevention should allow the capitalization of opportunities in using drugs with potential action to mature gametocytes (8-aminoquinolines and methylene blue) and to look for more innovative practices. Due to the lack of cost-effective treatments, future strategies complementing conventional malaria control tools (ITN, IRS, effective drugs) must include a malaria vaccine targeting all parasite stages and the threat of zoonotic infections through *P. Knowlesi.* It is likely to be crucial in reducing both the morbidity and the mortality of this disease if used in combination with existing therapeutic protocols. In particular, careful consideration should be given to the differences in the epidemiology of malaria between and within countries and health systems infrastructure. In particular, there should be an evaluation of the most accessible and demand driven channels, such as access to health care facilities, role of the private sector (informal vs. formal) in providing care, and availability of community-based health services. Except for the two MDG's on gender equality and environmental sustainability, six out of the eight MDG's will not be met if malaria is not adequately addressed with a visible and measurable impact. Achieving these goals would require resources, capacities and the use of evidence to drive policy.

As much as is possible, the national treatment guidelines need to be aligned with international recommendations but should also consider the in-country efficacy of an antimalarial, the readiness of the health staff to adopt new behaviour and the availability and the affordability of the recommended drugs. Non-adherence to guidelines is still a major problem which if not addressed will result in the appearance and spread of resistance. Whereas, the respect of guidelines helps sustain treatment regimens and preserve present molecules while giving proper basis for treatment policy change in case of resistance, frequent stock outs allow the rush in of sub standards. Quality Assurance is a crucial strategy in the fight against the appearance and spread of resistance. According to the QAMSA study (Quality of Anti-malaria Drugs in Sub-Saharan Africa), only 60% of currents drugs meet the standards while 40% are substandard or fakes. The quality of drugs was affected by two main factors - stock outs and the lack of regulatory control services. Consequently, the National Malaria Control Programme has as major roles the monitoring of the effectiveness of recommended treatments, the use of evidence to motivate policy, to inform health workers on the most recent guidelines and to supervise their implementation through periodic evaluations.

Résumé Analytique

L'Académie des Sciences du Cameroun a convoqué environ 60 chercheurs de différentes Universités et institutions de recherche du 31 janvier au 1er février 2013 à l'Hôtel Hilton de Yaoundé afin d'examiner la situation de résistance aux médicaments antipaludéens au Cameroun. L'atelier a été subdivisé en groupes de 4 à 5 chercheurs suivi de séances de restitution.

Dans les discussions qui ont suivi, il en est ressorti que la surutilisation des Combinaisons Thérapeutiques à base d'Artémisinine (CTA) au Cameroun peut aboutir à la résistance, surtout si elle n'est pas accompagnée par l'éducation adéquate sur les correspondances aux directives qui sont enfreintes. A cet effet, depuis 2002, la politique a changé et l'on est passé des monothérapies aux CTA en 2004. Artesunate-Amodiaquine (AQ) a été utilisé pour les traitements de première intention et Artéméter-Luméfantrine pour les traitements de seconde intention. En 2006, pour les cas de neuropaludisme, la quinine a été utilisée comme médicament de traitement alternatif en attendant l'administration de l'Artéméter injectable. Un consensus national s'est dégagé pour inclure les RTD pour les diagnostics en 2009 comme partie de l'algorithme de traitement. ASAQ, AL et dihydro-artémisinime-pipéraquine (DHAP) introduits sur le marché camerounais se sont avérés d'une efficacité comparable. Tel est également le cas pour l'efficacité *in vivo* du Sulfate de Quinine (SQ) pour les cas de paludisme non compliqué dont l'évaluation et la démonstration ont prouvé qu'il sévit à un taux de 94,2% dans la Région du Sud-Ouest du Cameroun. La surutilisation des CTA et de la quinine dans le secteur privé pouvant survenir surtout lorsque le traitement du paludisme est administré aux malades ne souffrant pas de paludisme (les malades exempts de parasite) peut aboutir à la sélection des parasites résistants. *Il est à cet effet nécessaire que le traitement se fonde sur la présence effective de Plasmodium spp détectable par des tests de diagnostic rapide (TDR) ou d'un microscope. Les institutions de formations en matière de santé doivent être mis à jour de ces directives.*

Les mutations sur les enzymes du métabolisme des médicaments ainsi que les marqueurs microsatellites qui bordent ces gènes représentent d'excellents marqueurs en sciences des laboratoires, en diversité génétique et en établissement des profils de résistances. Ceux-ci ont servi à la démonstration d'une représentation claire sur les lignées résistantes au Cameroun, avec les données de la région du Nord différentes de celles du Sud sur la base des marqueurs de résistance sur les gènes *dhps, dhfr, pfmdr1* et *pfcrt*. Les lignées résistantes de *dhps* du Cameroun, lorsque placées dans le contexte des autres pays africains, se rassemblaient seules comme une des 5 autres variantes, avec la conclusion que les lignées résistantes auraient pu survenir et se diffuser à différents moments en Afrique. Avec l'émergence de la résistance aux Artémisinines déjà déclarée en Asie du sud-est, *il nous est recommandé d'optimiser l'utilisation actuelle de médicaments et de développer de nouvelles formules allant dans le sens de l'éradication du paludisme*. Cela peut signifier que nous devons reprendre l'étude à zéro afin de redéfinir les mécanismes de résistance, les stratégies pharmacologiques et les présentations immunologiques des populations vulnérables. *Nous avons besoin des technologies abordables qui permettent de réaliser des diagnostics ultrasensible immédiats et sur site chez des centaines de malades. Continuer la politique des thérapies empiriques pourrait être rentable pour un moment mais pourrait aboutir de manière ultime à une augmentation à long terme de la résistance du parasite.* Le paludisme à *P. falciparum* grave peut être traité par 12 ou plusieurs autres thérapies et environ la moitié sont en phase de développement clinique. Cependant, le seul traitement pour les stades hépatique asexués et les stades sanguins sexués de toutes les espèces de primaquine et l'ajout d'une dose de primaquine à des régimes à base d'artémisinine peuvent aider à contrer la propagation de la résistance à l'artémisinine. La complexité du parasite du

paludisme lui-même et de son cycle de vie suppose que la fabrication d'un vaccin est plus problématique que pour les virus. En plus, certains chercheurs pensent qu'il est peu probable qu'une sous-unité d'une seule protéine recombinante ne puisse jamais fournir une protection adéquate afin d'être utilisée de manière générale.

Dans les approches de Santé Publique en matière de prévention du paludisme, il a été noté que le NMCP procède de deux manières - le contrôle du vecteur à travers l'utilisation de LLIN et IRS et la chimioprévention. Avec les diagnostics difficiles et les réactions inflammatoires dans le placenta, le paludisme au cours de la grossesse a des conséquences néfastes sur la mère et le fœtus. L'IPTp est efficace et réduit considérablement les proportions de parasitémie périphérique et placentaire. Par ailleurs, les études publiées démontrent que la couverture IPTp en traitement de paludisme chez les femmes enceintes doit être intensifiée à partir du très faible taux actuel qui est de 60%. La pharmacothérapie massive ou la chimioprévention représente une option de prévention de la transmission entre les saisons étant donné que ces thérapies sont considérées comme intervention de santé publique à une pandémie toujours croissante. *Les domaines d'action prioritaire identifiés comprennent l'utilisation rationnelle des médicaments par les populations vulnérables et la compréhension de la pharmacocinétique et de la pharmacodynamique de la pratique actuelle de la consommation des formules à base de plantes en association aux formules de médicaments existants*.

La perte des CTA comme traitement efficace contre le paludisme compromet gravement les efforts de lutter contre le paludisme et de l'éliminer complètement de l'Afrique Sub-saharienne. La propagation de ce parasite résistant en Afrique sub-saharienne constituera une catastrophe de santé publique. *A cet effet, l'utilisation rationnelle des CTA uniquement chez les patients souffrant de paludisme est essentiel*. Le traitement et la prévention doivent permettre de capitaliser les possibilités d'utilisation des médicaments ayant la capacité de mûrir les gamétocytes (8-aminoquinolines et bleu de méthylène) et à rechercher des pratiques plus innovantes. Du fait de l'absence des traitements moins coûteux, les stratégies futures venant en complémentarité aux outils conventionnels de lutte contre le paludisme (ITN, IRS, médicaments efficaces) doivent inclure un vaccin contre le paludisme visant tous les stades du parasite et la menace des infections zoonotiques à travers *P. knowlesi.* Il est probable qu'il soit crucial dans la réduction tant du taux de morbidité que de mortalité de cette maladie s'il est associé aux protocoles thérapeutiques existant. Surtout, une attention particulière doit être accordée aux différences épidémiologiques du paludisme entre et dans les pays, ainsi qu'au sein des infrastructures des systèmes de santé. En particulier, il faudra procéder à une évaluation des canaux les plus accessibles et à forte demande tels que l'accès aux structures de soins de santé, le rôle du secteur privé (informel vs formel) dans l'administration des soins de santé, et la disponibilité des services de santé communautaires. A l'exception des deux OMD portant sur l'égalité de genre et la protection de l'environnement, six sur huit OMD ne seront pas atteints si le problème que représente le paludisme n'est pas suffisamment abordé avec un impact visible et mesurable. L'atteinte de ces objectifs nécessitera des ressources, des capacités et l'utilisation des preuves pour mener cette politique.

Autant que possible, les directives nationales de traitement doivent être en phase avec les recommandations internationales, mais doivent également tenir compte de l'efficacité nationale des antipaludéens, de la volonté du personnel de santé d'adopter un nouveau comportement et de la disponibilité et de l'accessibilité financière des médicaments. La non-observance de ces directives constitue encore un problème majeur qui doit être résolu afin d'éviter l'apparition et la propagation de la résistance. Par contre, le respect des directives permet de faire durer les régimes de traitement et de préserver les molécules actuelles tout en donnant une base convenable à la modification de la politique de traitement en cas de résistance, la rupture fréquente de stock favorise l'entrée rapide des

produis de moindre qualité. L'Assurance Qualité est une stratégie cruciale dans la lutte contre l'apparition et la propagation de la résistance. Selon les résultats de l'étude QAMSA (Quality of Anti-malaria Drugs in Sub-Saharan Africa), seuls 60% des médicaments actuels sont conformes aux normes tandis que 40% sont de moindre qualité ou faux. La qualité des médicaments a été affectée par deux principaux facteurs - la rupture de stock et l'absence des services de contrôle de la réglementation. Par conséquent, le programme national de lutte contre le paludisme a pour rôles principaux, le suivi de l'efficacité des traitements recommandés, l'utilisation des preuves pour motiver la politique, pour informer le personnel de santé sur les directives les plus récentes et leur mise en application à travers des évaluations périodiques.

The Changing Nature of Malaria Management

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Introduction

Malaria is one of the greatest causes of mortality worldwide. Use of the most effective treatments for malaria remains inadequate for those in need, and there is concern over the emergence of resistance to these treatments. Yet the global distribution of medical schools and the world distribution of population and burden of disease are not well matched. Whereas world population is weighted towards Asia, the global burden of disease, measured in disability-adjusted life-years (DALYs), is heavily concentrated in Africa. The distribution of medical schools does not correspond well to either country population size or national disease burden [1].

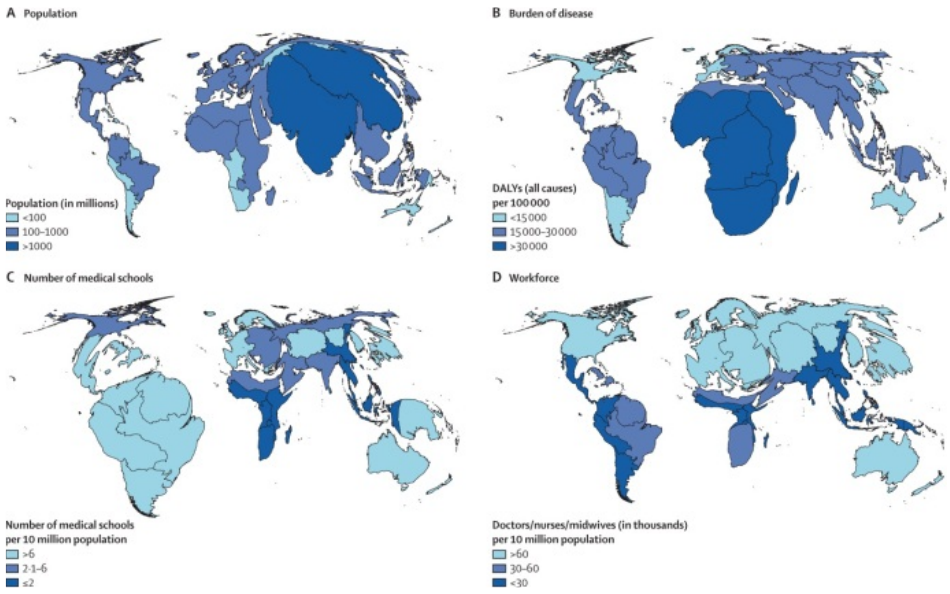


Fig 1. World maps resized by population (A), burden of disease (B), density of medical schools (C), and density of workforce (D)DALY=disability-adjusted life-years.[1]

Generally characterised by the fever and body chills and aches, malaria kills over one million people each year and its incidence is increasing largely attributed to falciparum. The mode of transmission of malaria has been known for more than 100 years. Despite this knowledge, malaria remains a serious public-health problem for roughly 40% of the world's population.It is caused by infection with a single-cell parasite, *Plasmodium*. Four *Plosmodium* spp cause malaria in human beings: *Plasmodium falciparum, P. vivax, P. ovaleP. malariae*. *P falciparum* is the most important because it accounts for the majority of infections and causes the most severe symptoms. A fifth form,*P. knowlesi,* the monkey parasite has increasingly been seen in man. Malaria needs not be a fatal disease and death can be avoided by prompt diagnosis and treatment.However, without this, a child may die of acute malaria within 24 hours. Transmission can be prevented by the use of bed-nets, repellents and insecticides.

The life cycle allows for the anopheline mosquito vectorto ingest erythrocytes infected with malaria gametocytes. Secondly,the gametocytes are released from erythrocytes and transformed into male and female gametes. In the third instance, zygotes are formed from the fertilisation of the female gamete by the male. Then the zygote develops into ookinete and penetrates the midgut wall of the mosquito. Fifthly, ookinete differentiates into oocyst and then ruptures releasing sporozoites that migrate to the mosquito's salivary glands. The sporozoites are then introduced into the intermediate host through the bite of an infected mosquito. These migrate towards the liver and invade hepatocytes and develop into schizonts containing thousands of merozoites. Alternatively, the sporozoites may remain latent in the liver as hypnozoites but then merozoites are released into the blood to infect circulating erythrocytes. In the blood cells, the parasite passes through the ring stage, thetrophozoite, andfinally the erythrocytic schizont is produced. The merozoites released, with the destructionof the erythrocytes begin the cycle again. Alternatively, some merozoites differentiate intogametocytes.

Treatment and Prevention

All the effective antimalarial drugs should kill developing *P. falciparum* gametocytes and all blood stages of the other human malaria species. Several of the available anti-malarials (e.g. antifolates and hydroxynaphthoquinones) also interfere with parasite development in the mosquito (sporozointocidal activity), but of these only the 8-aminoquinolines and methylene blue kill mature *P. falciparum* gametocytes. The ACTs have been demonstrated to do the same. The WHO advocates and encourages endemic countries to test, treat and track so that this drug can remain effective for much longer [2].

The cost of malaria is especially important in developing countries, because the economic effects of preventing and treating malaria are a large burden on limited health budgets and in view of many competing priorities. There is an acute need for cost-effective treatments to avoid the emergence of résistance. Asymptomatic asexual blood-stage malaria in zones that are marked for elimination might need some form of mass-drug administration. This problem applies especially to all of the malarias that are caused by hypnozoites and gametocytes. Yet to be able to eliminate malaria, presumptive therapy or mass-drug administration may require that all patients within a given area are treated for malaria irrespective of symptoms or diagnostics. This has been tried in school children in seasonal transmission zones and is called chemoprevention. Hypnozoite infection cannot now be diagnosed, and gametocytaemia presents all of the same diagnostic challenges of asexual parasitaemia. It is certain that the application of presumptive therapies and mass-drug administration requires more innovative and strategic thinking.

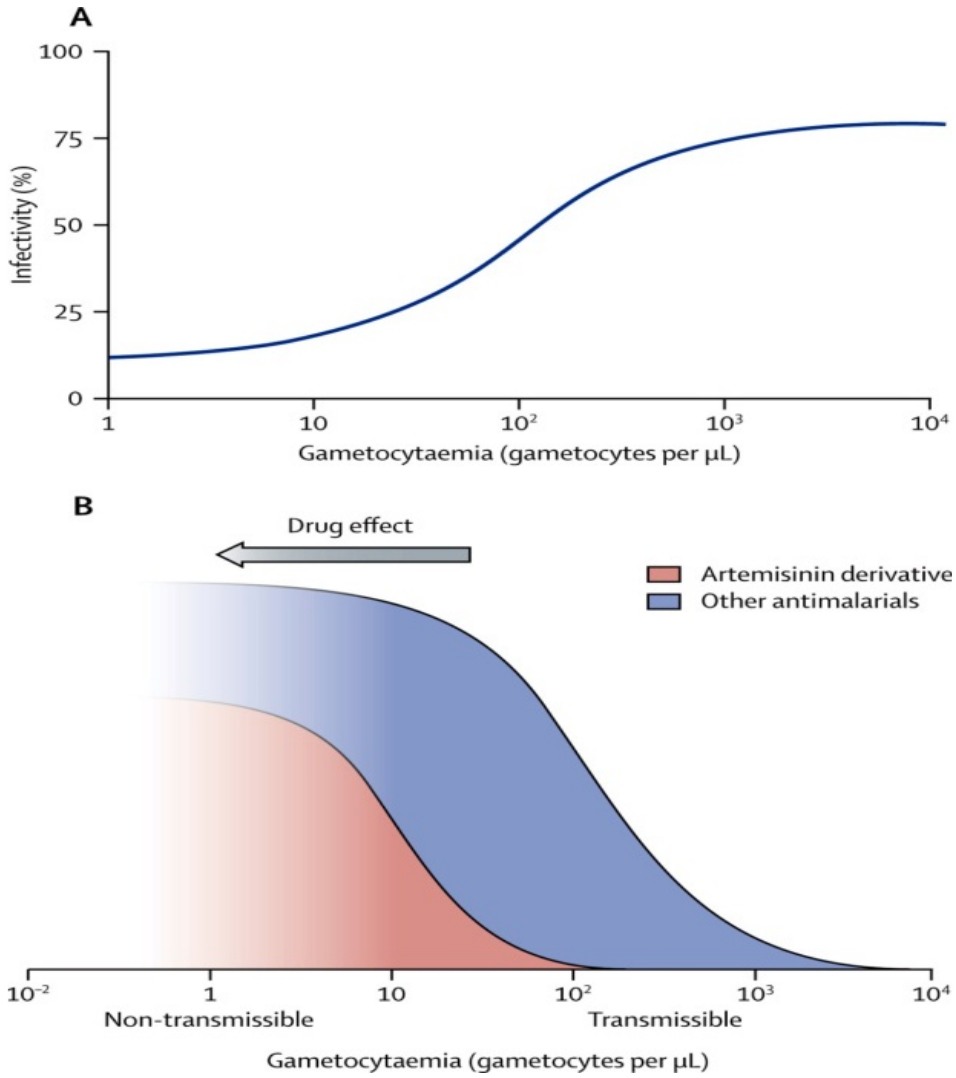


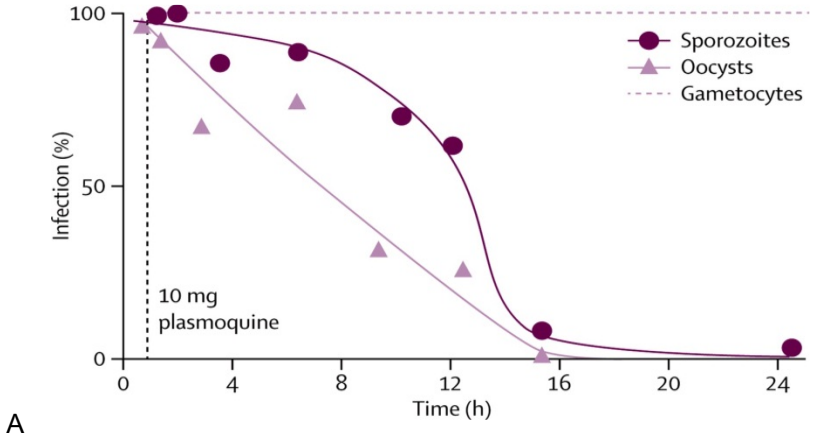
Fig 2. Effect of early anti-malarial treatment on transmissibility of falciparum malaria.

Early treatment aims to lower the number of viable gametocytes that enter the circulation and thereby reduce transmissibility. (A) Approximate sigmoid relation between P. falciparum gametocyte density and infectivity to anopheline mosquito vectors. (B) A rapidly effective treatment (e.g, an artemisinin derivative) prevents the maturation of gametocytes, which reduces gametocytaemia more than with other anti-malarials and thereby lessens transmissibility. Densities lower than ten gametocytes per µL become progressively less likely to be infectious, although substantial variability in infectivity is seen between individuals.

Artemisinin derivatives substantially reduce transmissibility in *falciparum* malaria largely by killing young gametocytes, but are less effective in patients who present with transmissible densities of infectious mature gametocytes.

The burden of malaria declined worldwide with increased deployment of insecticide-treated bed-nets and artemisinin-based combination therapies (ACTs). This observation caused many to entertain the idea that human malaria could eventually be eliminated. Although ACTs are the best treatment available for the species of parasite that causes the deadliest form of malaria, resistance to ACTs are feared in Southeast Asia. The spread of this resistant parasite to Sub-Saharan Africa would represent a public health catastrophe. Therefore, rational use of ACTs in patients with confirmed malaria is critical.

The ambitious but laudable goal that human malaria could eventually be eliminated is threatened by artemisinin resistance in *P. falciparum*. Artemisinin resistance increases treatment-failure rates. Slow parasite clearance is associated with increased gametocyte carriage, which provides a selective transmission advantage to resistant parasites, even without subsequent recrudescence, and thereby drives the spread of artemisinin resistance. The 8-aminoquinoline compounds possess unique gametocytocidal properties and rapidly sterilize the mature transmissible stages of *P. falciparum* [3]. MacKerras and Ercole[3] studied an adult volunteer with *falciparum* malaria and gametocytaemia who was bitten at frequent intervals by laboratory reared *Anopheles punctalus* before and after a single 10 mg dose of plasmoquine. The patient's initial count was 920 gametocytes per µL. Development of oocysts and sporozoites after treatment was rapidly inhibited, whereas gametocytaemia remained unchanged over 24H. On the other hand, treatment with Primaquine drastically reduced gametocytaemia.



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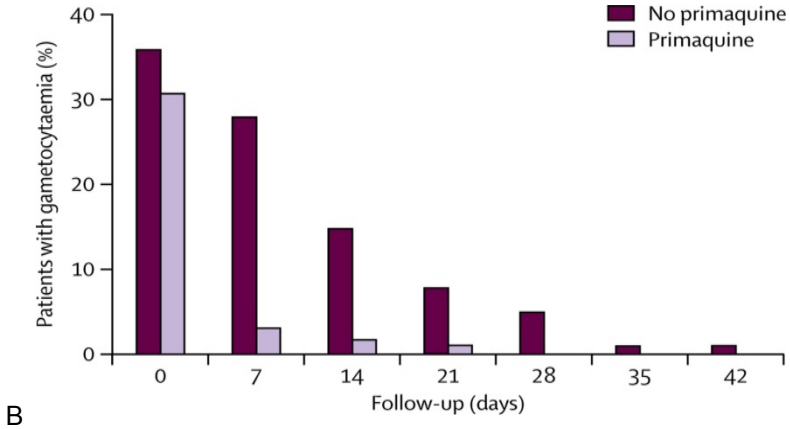

B

<u>Fig 3</u>. Comparison of gametocyte carriage in patients with falciparum malaria who did or did not receive primaquine

In a comparison of different artemisinin-based combination therapies in Myanmar (Burma), 808 patients were randomly assigned one of five different regimens, and half the patients were randomized to receive additional single-dose primaquine 0·75 mg base per 20 kg. Overall 264 patients presented with gametocytaemia as assessed by microscopy. Gametocyte clearance accelerated substantially after primaquine.

The addition of one dose of primaquine to artemisinin-based combination regimens could help to counter the spread of artemisinin resistance. Although primaquine is commonly recommended for *falciparum* and *vivax* malaria, concerns about drug-related haemolysis frequently prevent its administration. The limited available evidence on transmission-blocking effects of primaquine and its forerunner plasmoquine, suggests that doses lower than currently recommended (0·50 - 0·75 mg base per kg), which would be safer, might still be very effective [4].

Global Therapeutic Initiatives

Use of the most effective treatments for malaria remains inadequate for those in need, and there is concern over the emergence of resistance to these treatments. In sub-Saharan Africa, children under five in impoverished, rural areas are at the greatest risk of dying from malaria. One approach to improving the quality of malaria case management in the private sector is the Affordable Medicines Facility – malaria (AMFm), an innovative financing mechanism designed to save lives and delay the onset of resistance to artemisinin by expanding access to affordable artemisinin-based combination therapies (ACTs) for malaria through the public, private, and nongovernmental organization sectors. This is done through a factory-gate subsidy for ACTs that will reduce the price for local buyers and pass on those savings to the patient. The AMFm is hosted by the Global Fund and was launched in some African countries (*Ghana, Kenya, Madagascar, Niger, Nigeria, Uganda and Tanzania)* in 2009. The four stated objectives: increased ACT affordability, increased ACT availability, increased ACT use, including among vulnerable groups, and "crowding out" of oral artemisinin monotherapies, chloroquine and sulfadoxine-pyrimethamine by gaining market share [5]. AMFm involved manufacturer price negotiations, subsidies on the manufacturer price of each treatment purchased, and supporting interventions such as communications campaigns. The AMFm evaluation study demonstrated that subsidies combined with supporting interventions can be effective in rapidly improving availability, price and market share of QAACTs, particularly in the private for-profit sector. Decisions about the future of AMFm should also consider the effect on use in vulnerable populations, access to malaria diagnostics and cost-effectiveness [6].

The President's Malaria Initiative, USA is committed to working with the Global Fund to Fight AIDS, Tuberculosis and Malaria (Global Fund), and the World Health Organization (WHO), the Roll Back Malaria Partnership (RBM) and other partners to support evidence-based strategies to introduce malaria diagnostics, treatment and referral support for suspected severe febrile cases in children in the private sector, when endorsed by national malaria control programmes.

Future Perspectives and Challenges

The mainstays of malaria control are "bed nets, indoor residual spraying and anti-malarials", says Philip Bejon (Oxford University, UK), who has worked on several trials of the RTSS vaccine. These interventions are only partially effective. Even with the methods deployed maximally, most of Africa is likely to remain above the threshold for continued transmission. So there is a desperate need for something more.

The Challenges

1. The lack of cost-effective treatments and the emergence of resistance suggest a malaria vaccine is likely to be crucial in reducing both the morbidity and the mortality of this disease.
2. The need for an effective vaccine is greater than ever. A major difficulty with vaccine research is that the malaria parasite presents thousands of antigens to the human immune system that vary throughout its life cycle. Identifying those that may prove to be vaccine targets is complicated and time consuming.
3. Most vaccines are targeted at individual stages of the malaria life cycle, although it is likely that only the development of a multistage vaccine will offer complete protection to both visitors to, and residents of, a malaria-endemic area. With the development of a successful vaccine other issues such as cost, distribution, education and compliance will have to be addressed.

Concluding Remarks

1. The overuse of ACTs within the private sector that can occur when malaria treatment is provided to patients who do not have malaria can lead to the selection of resistance parasites. The loss of ACTs as an efficacious malaria treatment would severely jeopardize efforts to control and ultimately eliminate malaria in Sub-Saharan Africa. Spread of this resistant parasite to Sub-Saharan Africa would represent a public health catastrophe. Therefore rational use of ACTs only in patients with confirmed malaria is critical. Due to the lack of cost-effective treatments and the emergence of resistance, a malaria vaccine is likely to be crucial in reducing both the morbidity and the mortality of this disease.

2. We need affordable technologies that allow ultrasensitive same-day, on-site diagnostics in hundreds of patients. Pursuing the policy of presumptive therapies may be temporarily cost-effective but may ultimately lead to long-term increase in parasite resistance. Acute *P. falciparum* malaria might be treated with 12 or more therapies; half as many are in clinical development. However, the only treatment for the asexual liver stages and the sexual blood stages of all species is primaquine, the addition of one dose of primaquine to artemisinin-based combination regimens could help to counter the spread of artemisinin resistance. The complexity of the malaria parasite itself and its lifecycle mean that designing a vaccine is more problematic than for, say, viruses. And some researchers feel that a single recombinant protein subunit is unlikely to ever provide adequate protection to be of general use.

3. In particular, careful consideration should be given to differences in the epidemiology of malaria between and within countries, health systems infrastructure. In particular, there should be an evaluation of the most accessible and demand-driven channels, such as access to health care facilities, role of the private sector (informal vs. formal) in providing care, and availability of community-based health services

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Malaria and Development : The Millennium Development Goals

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Malaria kills 2,000 children every day and is directly linked to underdevelopment with the vicious cycle whereby it affects mostly the poor and is responsible for draining their meagre resources. The Millennium Development Goals (MDG's) seek to redress under development by improving on human wellness through the :

i. Eradication of extreme poverty and hunger;
ii. Achievement of universal primary education;
iii. Promotion of gender equality and empower women;
iv. Reduction of child mortality;
v. Improvement of maternal health;
vi. Fight against HIV/AIDS, malaria and other diseases;
vii. Safeguarding of environmental sustainability; and
viii. Development of a global partnership for development.

Except for the two MDG's on gender equality and environmental sustainability, six out of the eight MDG's will not be met if malaria is not adequately addressed with a visible and measurable impact. To achieve these goals would require resources, capacities and the use of evidence to drive policy. In seeking to address the **first MDG** which is the eradication of extreme poverty and hunger, interventions in malaria require prevention of infection involving the use of Insecticide treated nets (ITN's) for which comparative villages in Africa have shown reduction in episodes in those that use them (Barat, 2006; Bouyou-Akotet *et, al*, 2009). Environmental care is also important and must be encouraged. Early diagnosis, effective treatment and home management are essential. There is the need to reduce the over prescription of Artemisinin combined therapy (ACTs) through the use of diagnostics including microscopy and rapid diagnostic tests (RDT) to demonstrate the presence of parasites with a fever before treatment. All of the above put in place shall result in the reduction of cases of malaria, and when they do occur, put it under control so that much will be gained in time and money. Less malaria therefore will result in reduction in work time lost and also in money spent on health and therefore decrease in poverty level.

For the second MDG, the **rate** of school attendance and performance is directly tied to malaria infection. School attendance is affected negatively by malaria illness, also harbouring malaria parasite impacts on the school performance negatively. Anaemia from repeated episodes of malaria reduces the cognitive power of the children and all these giving rise to failure and high school dropout. School performance would therefore improve if there is a preventive sanitation, appropriate education to sleep under an ITN and a school based deparasitization of Malaria. Actually **i**ntermittent screening and treatment of malaria has been shown to be effective in reducing the infection rate and with improved school performance and outcome.

The **fourth MDG** seeks to **reduce child mortality**, and therefore requires the use of adequate and effective drugs, employment of intermittent preventive therapy in infants (IPTi) for which Fansidar-Amodiaquine combination (SPAQ) alongside the Expanded Program on Immunisation (EPI) has been shown to be protective in Senegal (Grobusch *et al*, 2007). Intermittent preventive therapy in school children (IPTc or IST) is protective by 85% using Fansidar and Artesunate (SPAS). Other formulations such as Amodiaquine-Artesunate are being tried in Ghana. Still in the prevention of infant mortality, the most advanced candidate malaria vaccine in clinical trials, the RTSS, was shown to reduce morbidity by 30-50% for up to 18 months (Alonso et al, 2004).

The **fifth MDG** seeks to improve on maternal health. Progress being made to achieve this includes the further development of an anti-disease vaccine with the identification and characterisation of VAR2CSA responsible for binding of parasites to the placenta. In Intermittent Preventive Therapy in pregnancy (IPTp) both SP and SPAQ are used. Also to be coupled to IPT is ensuring that pregnant women sleep under ITNs every night. These measures have been shown to reduce the negative effects of malaria in pregnancy and thereby improving maternal health.

The sixth MDG requires that we combat HIV/AIDS, malaria and other diseases. **The eighth MDG** is in the development of a global partnership for development. To begin to address these, many institutions and consortia have emerged - EDCTP, WHO (ADRN, MIM, TDR, RCS), NIH (FIC), GMP (EANMAT, WANMAT I & WANMAT II, SANMAT), RACTAP, IRD, EU (BioMALPAR, ANTIMAL, EUROMALVAC, AMANET), IAEA and BMGF (ACT, Vector, IPTi, INDEPTH – MCTA). The Roll Back Malaria Partnership is there to take the lead in coordinating efforts in implementing the Global Malaria Action Plan, the blue print to control and move malaria agenda towards its elimination.

The third MDG that encourages gender equality and the seventh MDG which encourages environmental sustainability do not need a malaria redress for those to be met.

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Malaria Epidemiology and Transmission Potential Post Long-lasting Insecticide-treated Nets in Cameroon

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Introduction

There has been a reduction of about 25-50% in reported malaria cases in most endemic countries between 2000 and 2010. About 80% of the estimated 216 million episodes of malaria reported in 2010, occurred in Africa. About 91% of the estimated 655,000 malaria deaths in 2010 were in Africa. This included 86% of global malaria deaths inchildren less than 5 years. The incidence of malaria has reduced by 17% and malaria-specific mortality rates by 26% since 2000. Even though this is a major achievement, the reductions are still less than 50% of the reduction targets for the target for 2010. In Cameroon malaria remains a huge public health challenge despite the efforts made to curb its spread. It accounts presently for :

- 40% of morbidity (38.8% in 2009);
- 36% of outpatient consultations;
- 38% of the cases in pregnant women (44.0% in2009);
- 48% of hospitalization (50.0% in 2009);
- 67% of deaths among children below 5 years (67.0% in 2009).

The most vulnerable groups are children below five years, pregnant women and non-immune persons/immigrants from non-malariazones. One may question why the huge burden? Answers may include poverty, political instability, human activities, efficient mosquito vectors, drug resistance, insecticide resistance and inadequate and inconsistent allocation of resources for control. These exacerbate the problem of malaria control, which is almost unattainable in most endemic areas. Endemicity in Cameroon is determined by the species of mosquito vectors involved in transmission, the parasite species in circulation, environmental and climatic conditions and human activities. Vectors are the key determinants of transmission with about 14 such vector species in Cameroon

Epidemiology of malaria

Based on the differences in environmental requirements for specific vector needs and transmission, Cameroon has 3 main eco-epidemiological zones for malaria (Mouchet *et al.*, 1993).Each is independently colonised by malaria vectors that vary with the type of environment. The eco-epidemiological zones for malaria transmission in Cameroon are the sudano-sahelian, the humid-savannah and the equatorial forest.

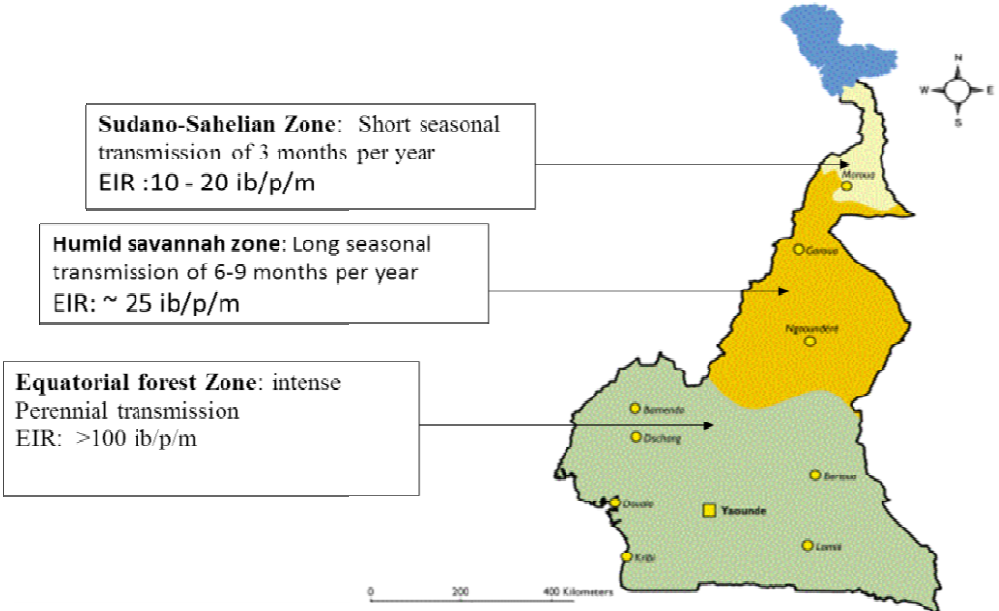


Figure 1.5: Map of Cameroon showing the eco epidemiological zones for malaria

(WHO/CAMINFOS, 2002)

The stratification of the eco-epidemiological zones for malaria in Cameroon demonstrates seven transmission patterns numbered I-VII.

The vector species by eco-epidemiological zones are classifiedas follows *I : sudano-sahelian zone; II : High inland plateau (Adamaoua) III : forest-Savannah transition zone; IV : Southern-forested equatorial zone; V : western highland / plateau zone; VI : coastal zone; VII : Urban zones*

The epidemiology of malaria is classified as stable or unstable malaria and zones without transmission. For the stable malaria zone, there is intense and permanent transmission (i.e. equatorial zones–bound to the rainfall, tropical zones) while for theunstable malaria zone, transmission is seasonal (i.e. savannas) and the zone is susceptible to epidemics or weak and episodic transmission (sahel, steppes, mountains).Areas without transmissionor moderate transmissionzones are susceptible to imported malaria.

Control and prevention

There are two main strategies used by the National Malaria Control Programme. The first is vector control that uses of long-lasting insecticide-treated nets (LLIN). From 2005 to 2009, more than 2 million nets targeting pregnant women and children less than 5 years old have distributed. There is a complimentary strategy to the first that has been using indoor residual spraying since 2007. The second strategy is chemoprophylaxis with intermittent preventive therapy in pregnancy that has been applied since 2004.

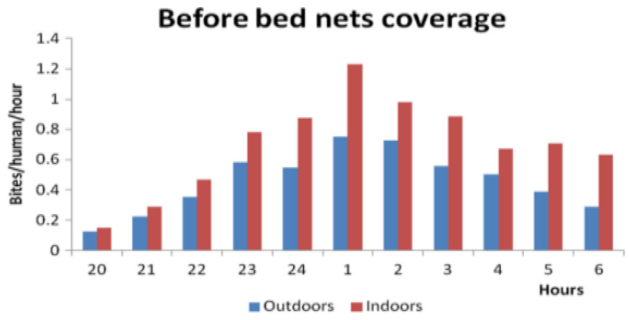
The effectiveness of malaria vector control using insecticide treated bed nets (ITNs) has been demonstrated in many studies.

The impact of the use of cyfluthrin (Solfac EW050) impregnated bed nets on malaria transmission in the city of Mbandjock was assessed by Antonio-Nkondjio et al.(2013). It was shown that the number of bites per person per hour and EIR reduced dramatically after the introduction of ITN. Similarly the number of species in circulation in Mbanjock aand Nkoteng seemed to have increased with the introduction of ITNs.

Table 2 Distribution of anopheline species collected in Mbandjock and Nkoteng from January 1997 to September 1998 before and after bed net distribution

	Mbandjock										Nkoteng	
	Before					After					Before	After
Species	Bilingue	Gare	Membrat	Plateau	Total (%)	Bilingue	Gare	Membrat	Plateau	Total (%)	n (%)	n (%)
An. coustani	0	3	0	0	3 (0.1%)	13	52	4	9	78 (2.4%)	0 (0%)	13 (1.63%)
An. funestus	66	77	99	48	290 (10.27)	31	48	27	44	150 (4.68%)	56 (41.48%)	472 (59.37%)
An. gambiae s. l.	431	612	977	113	2133 (75.6%)	389	1114	957	315	2775 (86.6%)	61 (45.19%)	274 (34.46%)
An. moucheti	62	30	27	27	146 (5.17%)	4	9	4	1	18 (0.56%)	2 (1.48%)	7 (0.88%)
An. ziemanni	3	27	3	4	37 (1.31%)	0	0	0	0	0 (0%)	6 (4.44%)	3 (0.38%)
An. nili	31	86	56	30	203 (7.2%)	19	119	18	29	185 (5.77%)	10 (7.4%)	22 (2.77%)
An. paludis	1	0	0	10	11 (0.39%)	0	0	0	0	0 (0%)	0 (0%)	4 (0.5%)
Total	594	835	1162	232	2823 (100%)	456	1342	1010	398	3206 (100%)	135 (100%)	795 (100%)

Before: Before bed net coverage; After: After bed net coverage; n: number collected; %: percentage.



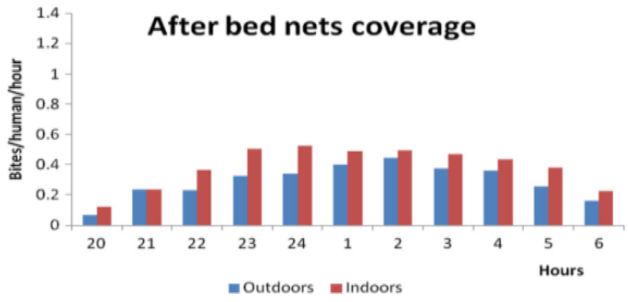
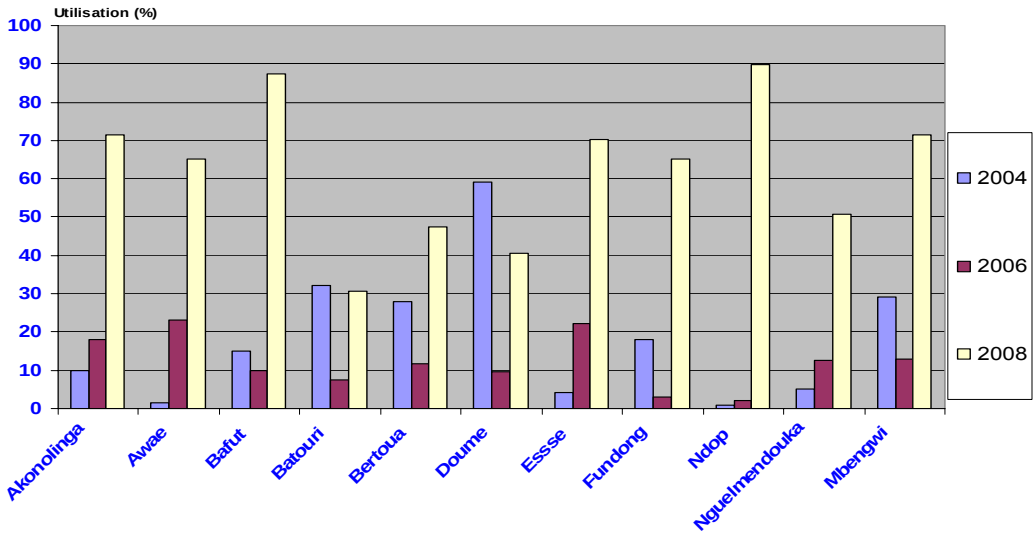


Table 6 Estimates of daily entomological inoculation rate (EIR)recorded in Nkoteng and districts of Mbandjock before and after bed net coverage

	Daily EIR		
Sites	Before bed nets	After bed nets	Reduction of EIR
Bilingue	0.464	0.066	85.86%
Gare	0.368	0.167	54.54%
Membrat	0.372	0.075	79.77%
Plateau	0.155	0.048	69.1%
Average Mbandjock	0.339	0.089	73.81%
Nkoteng	0.18	0.37	−105%

With the nation wide availability of ITNs for children less than 5 years old and more than 80% coverage, the situation may be more complicated than originally imagined. This may lead to changes in vector beheviour and resitance to insecticides.



There are several issues involved in the control of vectors with LLIN. These include the state of preparedness, the impact on burden/transmission, the possible occurrence of resistance to insecticides by the vector species, monitoring/evaluation strategiesand human behaviour. With over 8.7 million LLINs distributed in 2010 to ensure universal coverage across Cameroon, one may question what measures are taken to evaluate the effectiveness of LLINs on malaria burden and transmission. The WHO and BMGF project (IIR) in the north region of Cameroon is assessing the impact of LLIN on malaria disease burden and transmission.

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Evolution of Malaria Treatment Guidelines in Cameroon

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Introduction

Treatment guidelines aim at providing recommendations for the effective case management of the most vulnerable populations and therefore take all the relevant factors such as laboratory measures, drug efficacy results, antimalarial susceptibility to resistance and safety of the different antimalarial drugs in account[1].

Since the creation of the National Malaria Control Programme in 1998, national guidelines have moved from being orally disseminated to being written explicitly and focus on how to prevent and treat malaria cases. Following the reorganization of the National Roll Back Malaria Committee in 2002, efforts have been made to assemble useful data needed for the elaboration of treatment guidelines. Within the scope of the first National Malaria Control Strategic Plan 2002-2006, case management objectives were defined to take into account the epidemiological profile of malaria in Cameroon as provided by support strategies such as operational research. The level of endemicity in the country determined the diagnostic method adopted while the drug resistant profiles determined the treatment recommended.

These treatment guidelines have progressed over the years depending on the evolution of the above parameters. In general, the principles used in establishing treatment guidelines in Cameroon include: alignment to evidence-based recommendations provided by the World Health Organization (WHO), therapeutic efficacy test results obtained from within the country and the feasibility of applying the new treatment. When artemisinine-based combination therapies (ACT) were first introduced in 2004, the choice of drug was based on the efficacy of the partner drug which had to be above 95%. It is generally accepted that a treatment policy change is necessary when cure rate with the 1st line drug falls below 90%.Cost which initially constituted a major criterion in the selection of a treatment protocol is no longer a decisive factor given that antimalarial drugs benefit from national and international subsidies and donations.

Guidelines for the treatment of uncomplicated malaria

The choice of treatment for uncomplicated malaria is made based on the treatment objectives, which are to cure the infection, reduce transmission of the infection to others, prevent emergence and spread of resistance.

The evolution of the treatment of uncomplicated malaria can be described according to the periods in the history of malaria control in Cameroon:

[1] - *World Health Organisation 2010.*

Before 2002

Diagnosis was mostly presumptive and based on well-known clinical signs and symptoms in the absence of the danger signs of other focal points of infection. Microscopy was the only means of confirmation and was indicated in cases of treatment failure and severe malaria. This recommendation was based on the high malaria endemic state that made it highly probable for fever cases to be malaria. The first line treatment recommended was chloroquine while Amodiaquine or Sulfadoxine-Pyrimethamine was used as a second line drug[2].

2002 – 2004

Chloroquine resistance was first documented in Cameroon in 1985 in Limbe and later in other parts of the country. With the evolution of this resistance which varied between 2 and 66%[3], Amodiaquine and Sulfadoxine became first and second line treatment options respectively while artemisinine-based combination therapies began to appear on the market as possible alternatives[4].

2004 - 2006

In 2004, following WHO guidelines and in view of the increasing resistance to monotherapies including Amodiaquine and Sulfadoxine-pyrimethamin[5], there was a national consensus meeting on the selection of a first line drug during which ACTs were adopted in Cameroun and artesunate-amodiaquine selected as the first line ACT. Unfortunately, these drugsremained unavailable to the general public given their high prices and the insufficient knowledge of prescribers on ACT. In May 2006, the WHO released a press note on the withdrawal of monotherapies from countries experiencing antimalarial drug resistance. In response to that note, monotherapies were withdrawn and both artesunate-amodiaquine (ASAQ) and artemether-lumefantrine (AL) were retained as recommended treatments for simple malaria. These two ACTs were made available within the scope of grants from the Global Fund to fight against AIDS, Tuberculosis and Malaria (GFATM).

2008

Implementing a two-drug policy for simple malaria treatment proved to be very challenging due to unpredictable prescriber behaviour that led to inaccurate quantification. Non-adherence to national guidelines on ordering was a major drawback to this policy. These difficulties led to the revision of the national policy in 2008 which focused on the following aspects:

- presumptive diagnosis in children, pregnant women and in areas with difficult access to microscopy;
- systematic microscopy in persons above five;
- AS-AQ for subsidy;
- AL for public distribution without subsidy;
- quinine for pregnant women with simple malaria in 1st trimester and ACT as from the 2nd trimester.

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[2] - *Foreword of the First Malaria Treatment Guideline 2004*
[3] - *Basco et al., (Am J. Trop. Med. Hyg., 75(3), 2006*
[4] - *National Malaria Control Strategic Plan, 2002-2006*
[5] - *Mbacham et al., (Acta Tropica) 95S (2005) O-57 (S37)*

2009 - 2012

Access to universal diagnosis in target groups was limited given that microscopy demanded basic technical requirements including a qualified laboratory technician and power source. These resources were not available in most health facilities. In view of this, malaria rapid diagnostic tests (mRDTs) were adopted in 2009 and after 2 years of resource mobilization and procurement procedures, mRDTs were supplied to the Central Warehouse in 2011 and deployed in 52 pilot health districts, both at health facility and community levels. Lessons learnt during the pilot phase were used to scale up distribution to the entire country as from the year 2012 and systematic diagnosis of all suspected cases of malaria was enforced.

Guidelines for the treatment of severe malaria

The guidelines for the management of severe malaria are established based on key treatment objectives that include the need to prevent death, recrudescence and neurological sequels.

Before the 2006 revision of malaria treatment guidelines, intravenous and intramuscular quinine had been the principal drug. It was recommended to give 25mg/Kg body weight in 2 – 3 daily doses until improvement of symptoms and then to continue with a full oral treatment as in the case of simple malaria. The alternative for this treatment especially in cases of quinine intolerance or contraindication was injectable artemether using the 5-day protocol. Adjuvant treatment was advised for all severe symptoms. In 2006, quinine remained the recommended treatment but a loading dose of 20mg/Kg body weight was recommended to accelerate remission. Intramuscular artemether was an acceptable option provided it was administered using the 7-day protocol.

These guidelines were confirmed over the following 6 years until 2012, when in view of global evidence on the efficacy of injectable artesunate, this drug was introduced into the National Malaria Control Strategic Plan. Injectable artesunate was finally adopted by national experts in 2013 and its introduction has been planned for the same year in a number of pilot sites.

During pregnancy, all cases of malaria are treated as severe beginning with quinine infusions in the first trimester and then any of the other options (quinine, IV artemether or IV artesunate) as from the second trimester. IV artesunate always has priority when the indication is present.

Adherence to guidelines

The National Malaria Control Program measures adherence to national treatment guidelines using indicators that are calculated from the national health information system on malaria. Based on the number of confirmed or suspected malaria cases and the number that are treated using the recommended drug, there has been significant progress in the level of adherence.

Key indicators related to treatment

Year	2007	2008	2009	2010
Proportion of children under five who are correctly treated for simple malaria in health facilities	30%	57,90%	61%	67,40%[6]
Proportion of persons above five who are correctly treated for simple malaria in health facilities	30%	56,80%	60%	64,60%[6]
Proportion of children who are correctly treated for simple malaria within 24 hours in community	2.4%[7]	NA	NA	6%[8]

It is evident from the figures in the table that there has been a steady improvement in health workers' adherence to national malaria treatment policy. Among the factors that contributed to this situation are two trainings carried out in 2007 and 2009, increased availability and affordability of recommended ACT, sensitization and supervision carried out within the framework of the Global Fund grants. On the other hand, prompt effective treatment still remains a challenge. Accessibility to health facilities, slow uptake of community based interventions and lack of correct information in the general population explains why most patients still seek treatment out of the health facilities and usually do this later than the first day after onset of illness.

In conclusion, as much as is possible the national treatment guidelines are being aligned with international recommendations but are also influenced by the in-country efficacy of antimalarials, the readiness of the health staff to adopt new behaviour and the availability and the affordability of the recommended drugs. Non-adherence to guidelines is still a major problem which if not addressed will result in the appearance and spread of resistance. On the other hand, the respect of guidelines helps sustain treatment regimens and preserve present molecules while giving proper basis for treatment policy change in case of resistance. The National Malaria Control Program has as role to monitor the effectiveness of recommended treatments and use evidence to motivate policy, to inform health workers on the most recent guidelines and to monitor their implementation through supervision and periodic evaluations.

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[6] - *National Health Information System*
[7] - *Malaria baseline Survey*
[8] - *Demographic Health Survey*

Patient exit pool and provider practices : research on the economics of ACTS

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ScD, FASI, FCAS

Patients Appreciation of Treatment

The REACTs project investigated patients' knowledge on malaria treatment and compared malaria cases management in public and private health facilities in Yaoundé (Centre region of Cameroon) and Bamenda (North-West region of Cameroon).A sample of febrile patients from public and private health facilities indicated that very few people knew how to take the correct dose for malaria treatment (13.2% and 8.9% in public and private sectors respectively) (Figure 1).

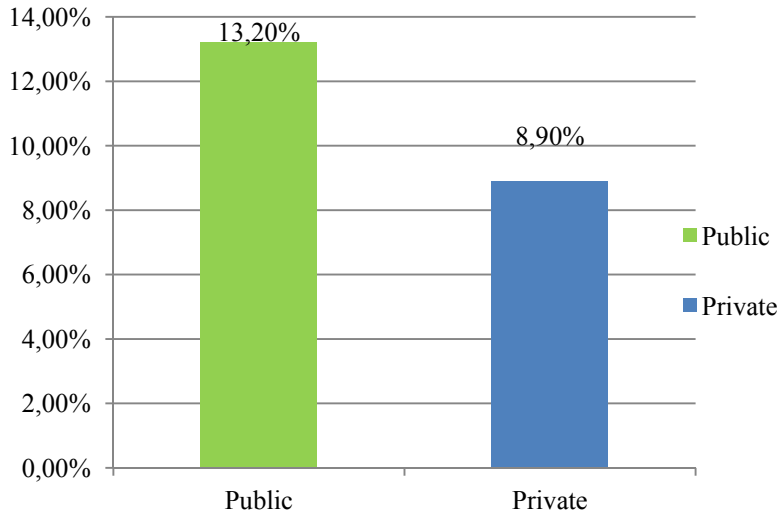

Fig 1. Percentages of febrile patients that received nationally recommended first line drugs for uncomplicated malaria in public and private sectors and knew how to take the medicine (Mbacham et al. 2010)

Treatment received

About malaria diagnosis and treatment, it was found that the proportions of people from both public and private vary with a slight advantage for the private sector. This concerns proportions of people that were diagnosed with malaria, which had a diagnostic test, which received any ACT, which received ACT in correct dose and patient knowledge on treatment regimen. But the tendency changes when we examine the proportion of patients that received national recommended ACT (now in the advantage of public sector).

Of those who were diagnosed with malaria without a diagnostic test, the proportion of patients that were attended with ACT in the correct dose is higher than 70 % in both sectors with a slight advantage for the private sector. This tendency remains the same when patients' knowledge about treatment regimen and correct dose were sampled.

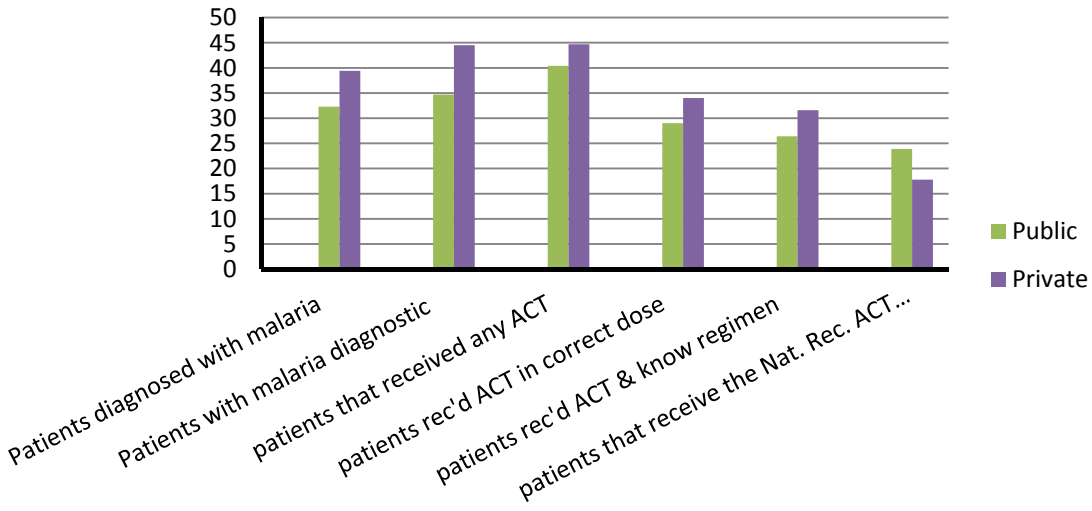

Fig. 2: Dose regimen of ACTs and evaluation of the knowledge on treatment received (Mbachamet al. 2010)
(Source : Mbacham et al. 2010)

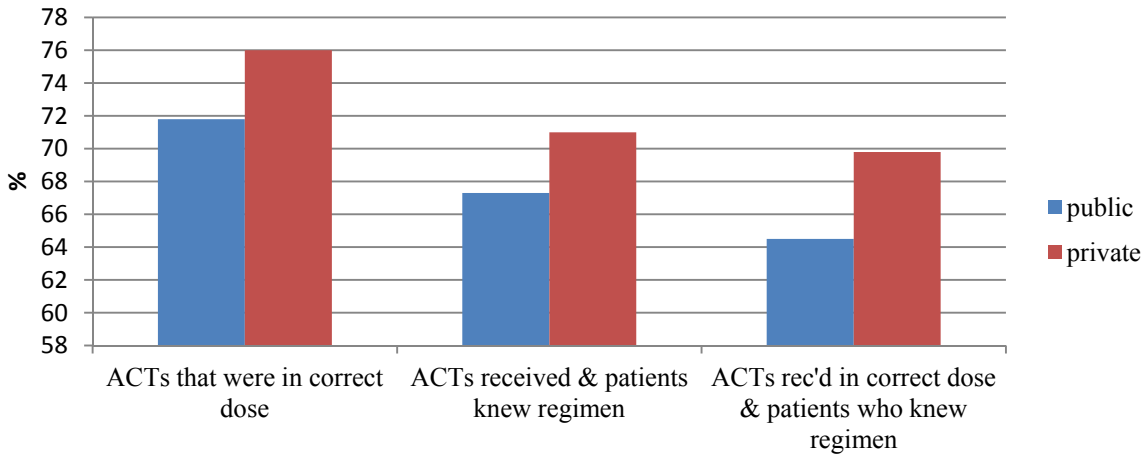

Fig. 2. Malaria diagnosis and treatment received by patients from Public and private sectors

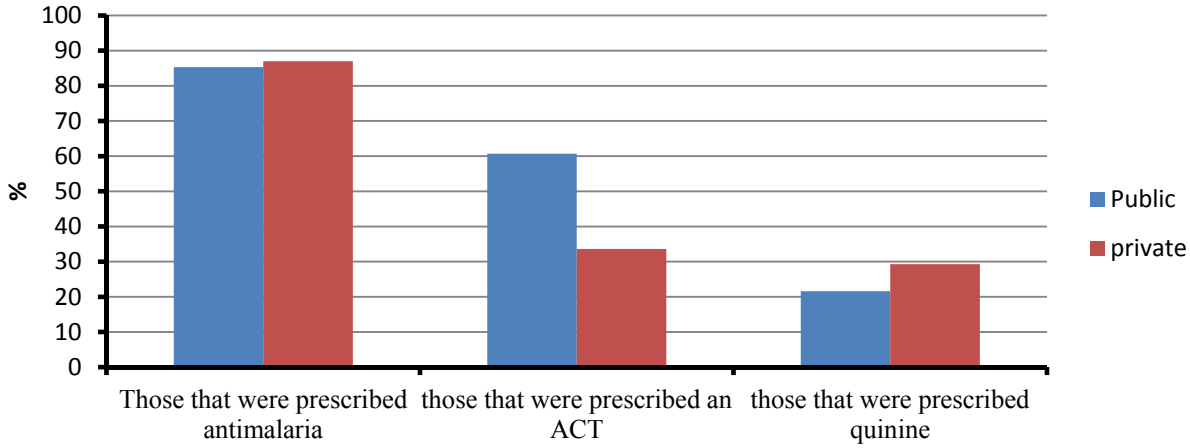

Fig.3. Treatment prescribed following malaria test for patients that reported having a positive malaria test

Treatment prescribed following malaria test

More than 70% of people with a negative malaria test were prescribed a malaria drug in both sectors. 10% of these people were prescribed quinine from private sector

More than 80% of the people diagnosed with malaria after a diagnostic test were prescribed and anti malaria drug. But only 30% of them received ACT from the private sector whereas 60% were put on ACT in public hospital (Figure 46).

Here the drugs prescribed by health workers (HWs) were the first and second line recommended drugs (AS-AQ and AL respectively) in both private and public sectors. In the public sector, the percentage of HWs who reported AL and AS/AQ as the national recommended treatment was greater (78%) than in private sector (Figure 5). In the private sector most workers reported AL as the national recommended treatment and knew the regimen for AS/AQ in an adult. Following these results, we are tempted to say AL is mostly subsidized by or through the private sector.

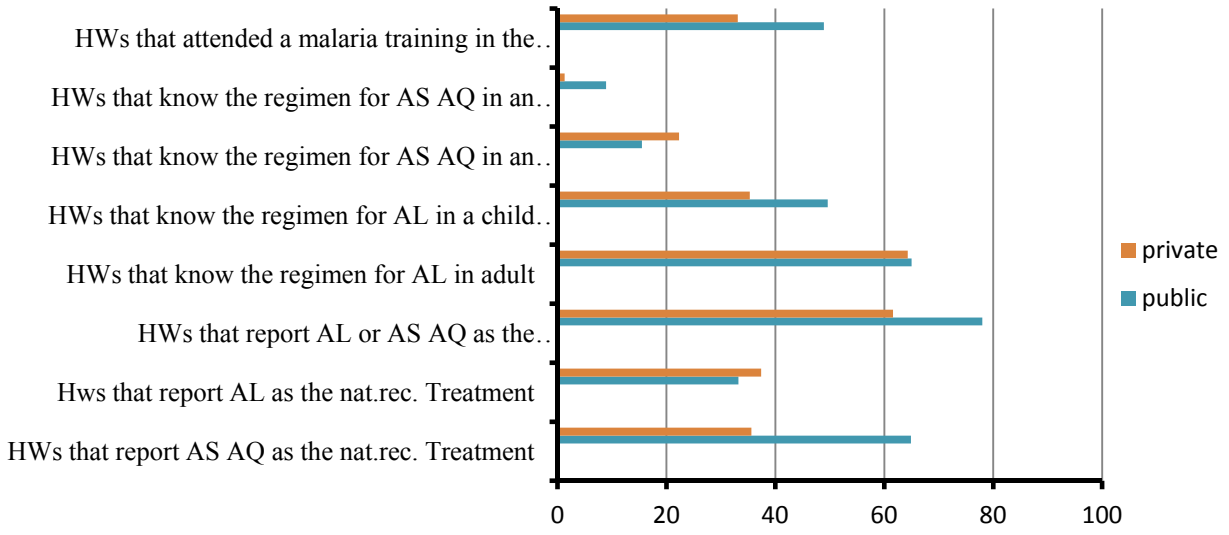

Fig. 3 : Health workers knowledge on ACTs used as 1st and 2nd line treatments in Cameroon

Health facilities outcomes

As stated earlier, there are many actors both from the public and private sectors that intervene in the deployment of ACTs in Cameroon. Following the graph below it may be thought that, health facilities in the private sector are more active in the deployment of ACTs or that they are less provided with ACTs than the public sector, which actually has various and different ACTs combinations in stock. Or in another point of view, it may be said that in public health facilities microscopy testing doesn't actually corroborate with the treatment to be given (high stock compared to private).

Access to and delivery of malaria treatment in Cameroon

The Cameroon government has issued guidelines for the rational use of ACTs in order to prevent development of resistance. According to these guidelines, patients can have access to subsidized ACTs from two sources: community relays and from the health facilities. A number of conditions for benefiting from these drugs have been published these stipulate among others that patients should have been consulted by a qualified health personnel in a health unit dispensing ACTs and hold a prescription properly filled and signed by a doctor. As for community relay where logistics are often limited, delivery of ACTs is done after observation of clinical signs associated with malaria in a patient and if possible a rapid test should be carried out. The average availability of ACTs across the national territory stood at 87.79%, in 2008 (NMCP, 2008). Problems had been identified as hindrance to drugs delivery: the poor road infrastructure network and the financial inaccessibility of the drugs even at subsidized prices to many populations. As a result many resort to traditional medicines or get cheaper but fake drugs from unauthorized outlets (NMCP, 2008).

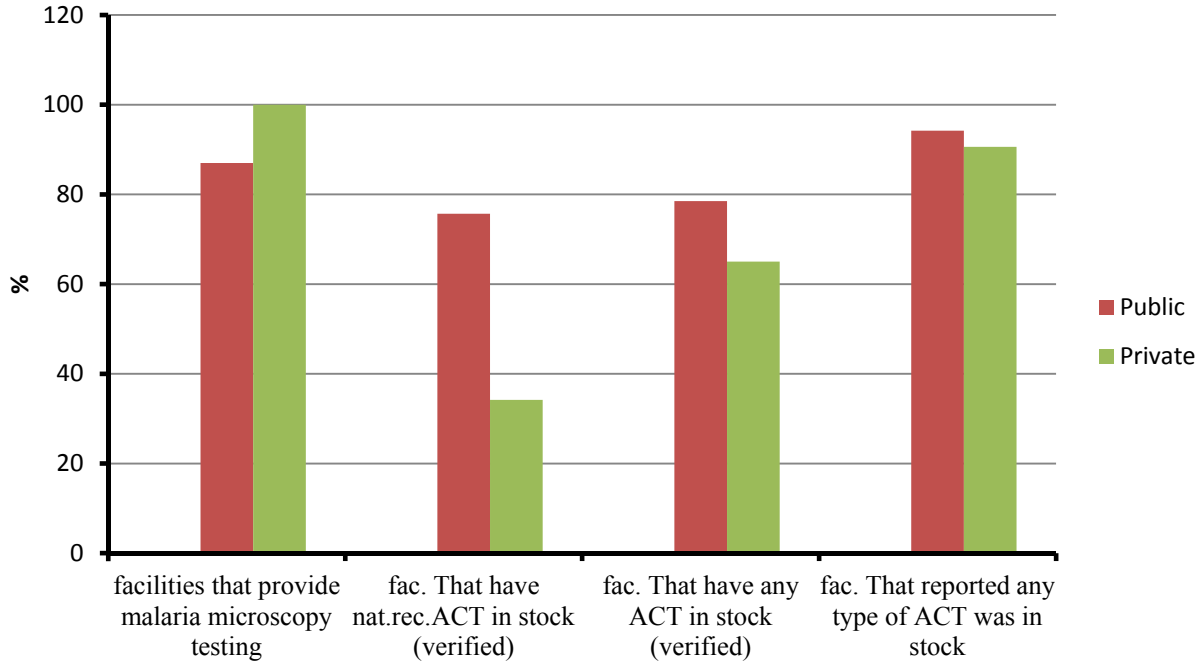

***Fig. 4* : Health facilities outcome in term of availability of ACTs and microscopy test (Mbacham et al., 2010)**

Treatment practices of providers

Adherence to guidelines for prescribing ACTs to patients depends on individual providers. Ongolo-zogo and colleagues (2008) reported that the reasons for violation of the guidelines are : the lucrative aspect of other treatments, the drugs stock and shortages of national recommended drugs, marketing pressure and a slow administrative system. Some providers said they only respond to patients' demand. Between 2006 and 2007, 70% of all children with fever were treated with anti-malaria drugs and 40% of which took it on the same day or the following day and none were given ACTs (INS, 2006). In 2008, a nationwide study on malaria treatment using ACTs for children under five and above five years was done by the NMCP and showed discrepancies among regions.

Proportion of patients who were prescribed/received the correct dose and advice on the regimen

According to MIC III (2006), Artesunate-Amodiaquine combination was scarcely used in the treatment of malaria (1%) for children less than five years who had a fever. The situation was worse in the North and Far North regions, where only 32% and 34% of children were appropriately treated and 9% and 22% in the 24 hours following the appearance of the first symptoms respectively for children aged less than five years.

Variations in service delivery

The quality of service delivery to patient varies from one provider to the other. Many patients do refer to confessional and private health facilities despite the price margin of drugs. They find them more comfortable and deem the services to be of higher quality when compared to public facilities. Like health facility preferences, the quality of treatment received also varies from one population group to the other; urban dwellers receive their first dose of treatment earlier after the appearance of the symptoms than the rural dwellers do; only 29% of rural children received an anti-malarial in the 24 hours following the symptoms whereas up to 53% of urban children do so *(Kiawi et al., 1997; Yomi and Koumaga, 2001; INS, 2006).* In addition, reference delay to health centres in rural areas is estimated on average to be 3 days. The reasons for this delay are underestimation of the disease, auto medication with medicinal plants or from family pharmacy box, absence of finance and distance to health facilities *(Ongolo-Zogo et al.*, 2008). Disparities in access to care are also related to the absence of dialogue between the health care provider and the patients *(Ongolo-Zogo et al., 2008*). It was noted that the educational level of mothers has no influence on the prevalence of fever in the child (*Kiawi et al., 1997; INS, 2006).*

Knowledge gaps

- Data on the incidence of fever in persons other than pregnant women and children under the age of five;
- Data on the number of people seeking treatment at the formal sector, who took medicines or an anti-malarial of a type before coming to the hospital.
- Data on the correct dose and advice on the regimen.
- Knowledge on the successes and failures of the intervention (NMCP, 2008) designed by the National Malaria Control Programme on improving access to and delivery of ACTs in Cameroon.

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Malaria in Pregnancy and the Intermittent Preventive Treatment

Prof Rose Gana Fomban-LEKE[9] and Prof Diane Wallace TAYLOR[10]

Background

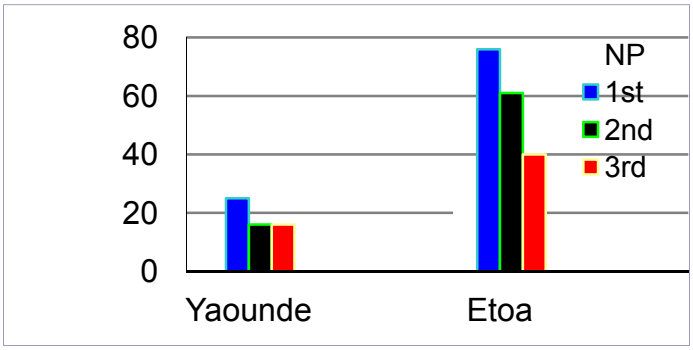
Worldwide malaria situation in pregnant women

Each year, 25 million pregnant women in Sub-Saharan Africa run the risk of contracting malaria. Infected women are at risk of severe anaemia resulting in significant impairment of foetal growth, prematurity and low birth weight babies. Maternal malaria may therefore be the cause of the death of 100,000 - 200,000 new born babies and 10,000 women each year. In Cameroon like most endemic countries, the prevalence of pregnancy associated malaria is higher in:

- Pregnant than in non-pregnant women,
- Primigravidae than in multigravidae because of pregnancy-associated immunity,
- Younger women due to the age-dependent immunity, and
- Women living in rural areas than those in the city due to the lack of health services.

The Key factors associated with susceptibility of pregnant women to malaria are therefore: the age of the woman, the gestational age, the parity, the level of endemicity and exposure.

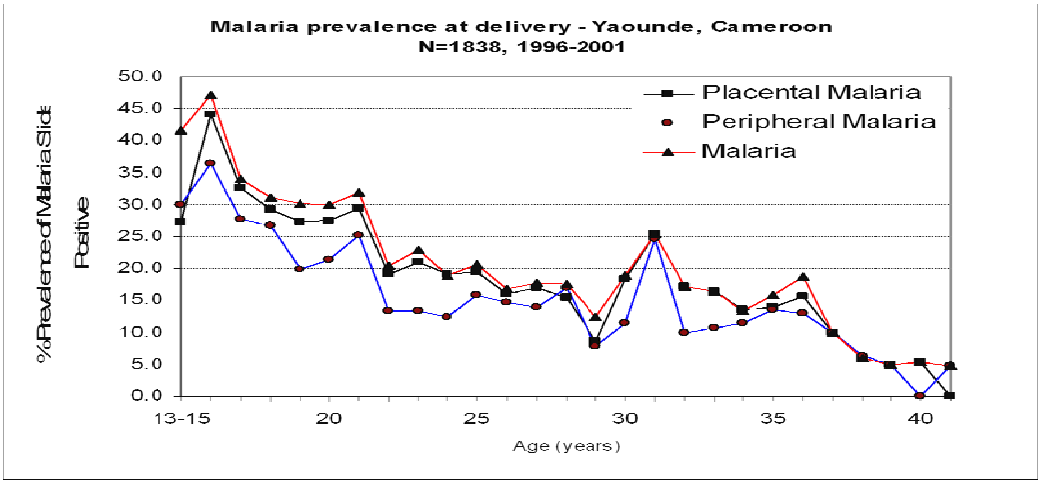
In Cameroon,*P. falciparum* is transmitted throughout the year with 2 wet and 2 dry seasons. In Yaoundé, it is estimated to have 13 infectious bites per year (Manga et al., 1992), whereas in Etoa, it is 2.4 infectious bites per night (Quakyi et al., 2000). This is reflected in the prevalence of slide-positive malaria in non-pregnant and pregnant women in the city of Yaounde (13 infectious bites per year) and the village of Etoa (~250 infectious bites per year).



Source : Zhou, Megnekou et al. 2002

The prevalence of malaria at delivery decreases with age in women living in Yaoundé (n = 1898 deliveries) as demonstrated in the fig below.

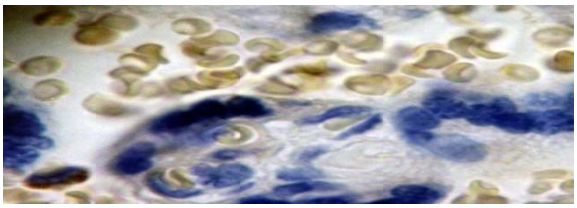
[9] - *Biotechnology Centre, University of Yaoundé I, Yaoundé, Cameroon.*
[10] - *University of Hawaii, USA*



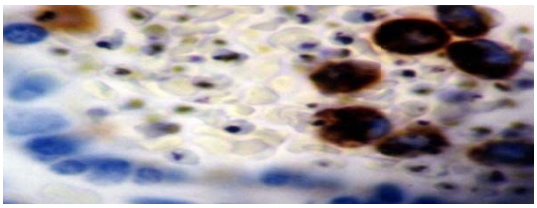
Source: Tako et al. (2003)

Diagnosis of placenta malaria

The diagnosis of *P. falciparum* infection is usually difficult in pregnant women. This is due to the fact that infected erythrocytes accumulate in the placenta and may not be detected in peripheral blood smears. Studies by the Leke group showed that at delivery, 21% of women that had parasites in the placenta were blood smear negative. This means that 1 out of five women with malaria during pregnancy is misdiagnosed. The advent of rapid diagnostic tests that detect histidine rich protein 2 has greatly improved the diagnosis of malaria in pregnant women, thereby allowing them to receive correct treatment.



Non-Infected Placenta



Malaria-Infected Placenta

Placenta malaria inflammatory responses

P. falciparum parasites sequester in the placenta due to the presence of ligands on parasites. There is monocyte infiltration into the intervillous space (IVS). Maternal WBCs in the IVS secrete chemokines and cytokines. Maternal monocytes and macrophages from infected placentas secrete more β-chemokines (e.g. MCP-1, MIP-1α, MIP-1β) that attract more inflammatory cells to the site. Foetal trophoblasts secrete IFNγ but not the other types of cytokines, in response to malarial parasite antigens (Suguitan et al., 2003). Studies on placenta malaria and its association with poor pregnancy outcome demonstrate that maternal monocytes/macrophages respond to the presence of infected RBCs in IVS by secreting cytokines (TNFa and IL-10) and chemokines. These chemokines attract additional macrophages to the placenta, kill parasites and cause pathology that increases the risk of poor pregnancy outcome. The association of low birth weight with TNFa and IL-10 is now well substantiated and hopefully drugs that can neutralise the effects of TNFa may be a way to prevent pathology when pregnant women are infected with drug-resistant malaria. The presence of malarial

parasites in the intervillous space (IVS) of the placenta alters the cytokine balance. An altered balance is associated with a poor pregnancy outcome.

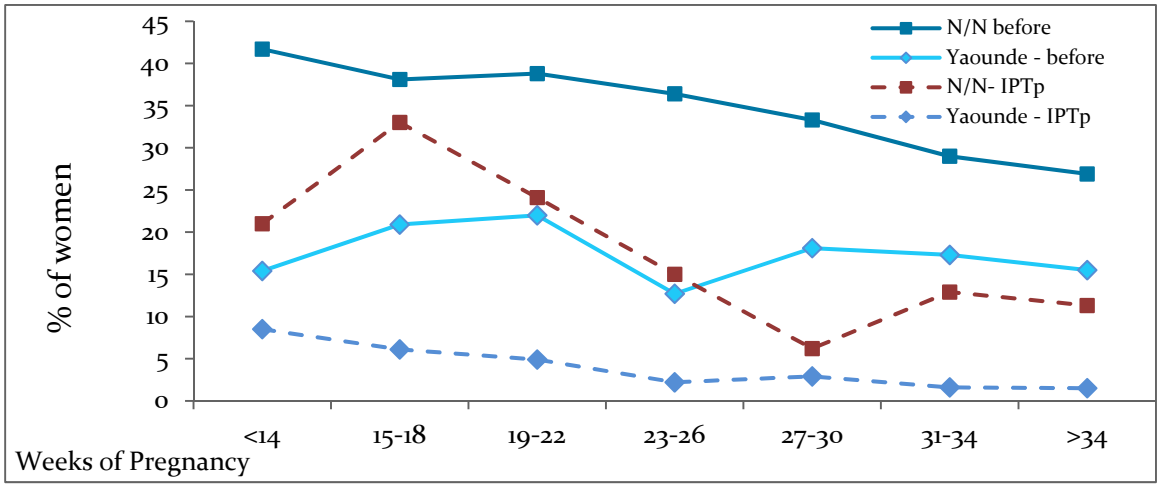
Women with anaemia and placental parasitaemias greater than 1% had the highest risk for Pre- Term Delivery (PTD). The other risk factors for PTD are anaemia, elevated IL-10 and a high IL-10 : TNF-alpha ratio (Suguitan et al., 2003). We can logically conclude that pregnant women are more susceptible to malaria and the parasites sequester in the placenta and induce the secretion of cytokines that can be associated with pathology and poor pregnancy outcomes. Sequestration of the parasites makes diagnosis of malaria during pregnancy difficult. Overall, 1 out of every 4 to 5 pregnant women with placental parasites is peripheral blood smear negative.

		No. of Pregnancies		
	MAL	1st	2nd	3+
Anaemia	POS	31.6	28.8	29.8
	NEG	15.7	16.7	15.3
Low Birth Weight	POS	28.0	20.6	9.9
	NEG	14.7	18.9	11.1
Pre-Term Delivery	POS	32.8	24.3	14.4
	NEG	16.4	20.9	17.4

Source: Tako et al., 2005

Policy for IPTp

A consensus meeting in 2002 in Cameroon recommended the Intermittent Preventive Treatment (chemoprevention) for Pregnant women (IPTp) at a dose of Sulphadoxine-Pyrimethamine given in the second trimester, then quickening the second dose at least a month later and a third dose at least another month later. The Malaria Policy and Advisory Committee (MPAC) recently recommended that after quickening, a dose at every pre-natal visit. There are normally 4 such prescribed prenatal visits. In our research we sought therefore to establish whether IPTp reduced the prevalence of placental malaria? In the experimental design, we looked at information before the advent of IPTp in Cameroon from 2001 – 2005 in an urban setting – Yaoundé and two other villages (rural) around Yaoundé. Then from 2007 to the present day, we have been monitoring the outcome and time to infection of these women.

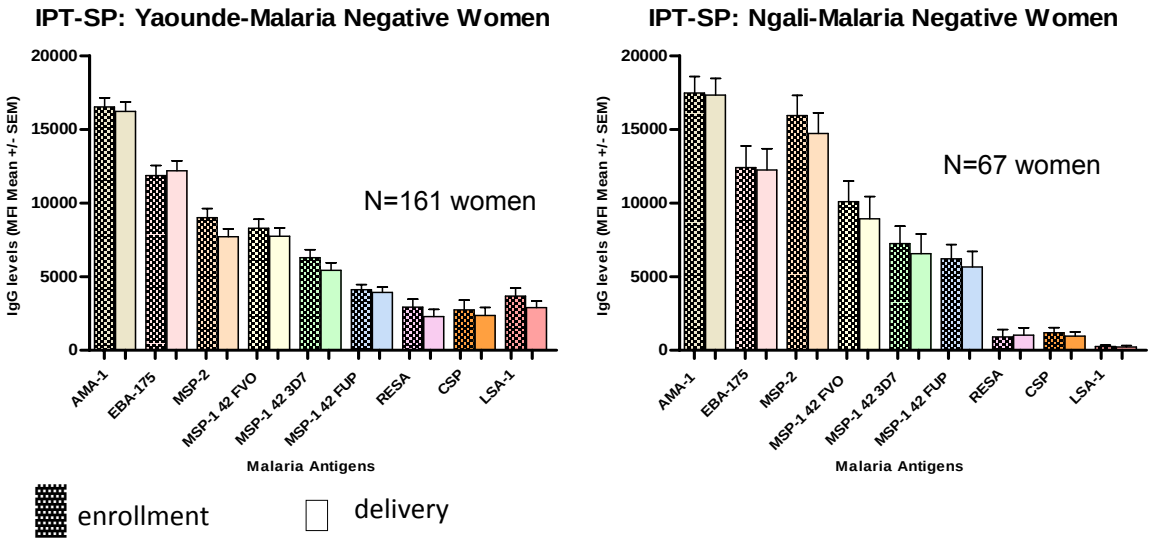


Results demonstrate that the administration of IPTp greatly reduced the prevalence of *P. falciparum* both in the urban (Yaoundé) and rural settings (Ngali and Ntoue).

Prevalence of *P. falciparum* malaria

Comparison of prevalence of malaria before and after IPTp implementation (Longitudinal Analysis)					
	City Yaoundé			Villages Ngali + Ntoue.	
	Before	IPTp		Before	IPTp
% women who became slide-pos at least 1 time during pregnancy	**61%**	**9%***		**80%**	**44%***
% who became anemic at least once	22%	12%		27%	33%
* Some of the women were slide-positive upon enrolment					

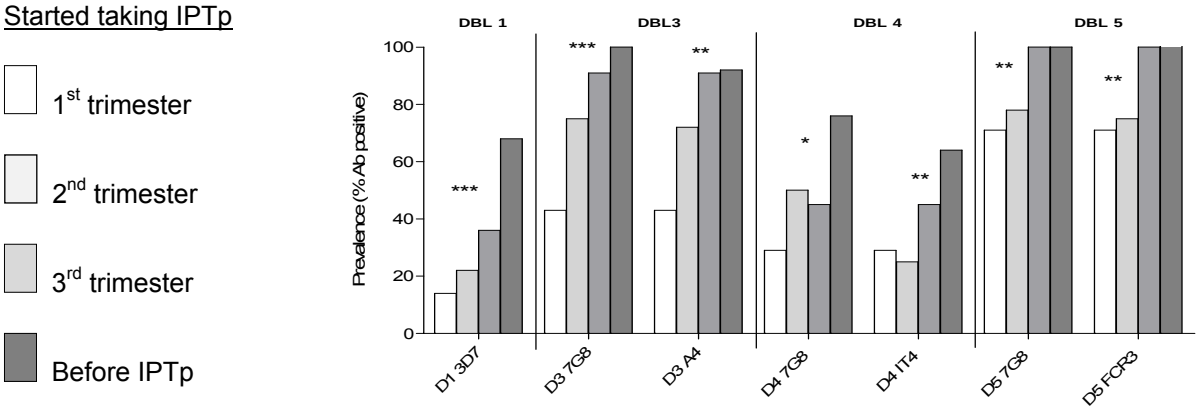
When antibody levels were compared, there was no significant reduction in antibody levels in women taking IPTp.



The Ab levels against VAR2-CSA (variable circumsporozoite surface antigen) were also measured. This is the molecule that binds to the trophoblasts and which has 6 Duffy-like (DBL) domains, at least 4 of which are involved in this interaction. Ab measured against DBL 1, 3, 4, 5, and 6 recombinant proteins, graciously provided by J. Smith, SBRI, Seattle, WA; A. Salanti, Univ. Copenhagen; and K. Singh, NIAID, NIH (*DBL 2 is difficult to clone and express and was not available for use in this study). Results show that there was an Influence of IPTp on the proportion of women who had Ab at delivery.

IPTp coverage in Cameroon

The IPTp coverage in Cameroon is generally very low (ranging from 20% to - 60%). The policy was adopted for 3 doses and yet the document now states that it is “at least two doses”. It is hoped that a new policy will soon be applied so that more women can benefit from it. There is the constraint that increasingly resistance is being developed towards SP and may jeopardize this approach.



The conclusions arrived at was that women taking ITPp had lower Ab levels at delivery to DBL 1, 3, 4, 5, but not DBL6, than women who did not take IPT (i.e., the before group). The longer they received IPTp, the lower the levels at delivery.

Conclusion

Malaria during pregnancy has deleterious consequences for the mother and the foetus. Diagnosis is difficult and there areinflammatory reactions in the placenta. IPTp does work and greatly reduces peripheral and placental parasitaemia. The new policy should be implemented and enforced in Cameroon

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Methods in Malaria Research - Efficacy and Resistance : In Vitro Evidence of Resistance

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Introduction

Malaria remains a public health burden in Cameroon with children and pregnant women at particularly high risk. Differences in parasite population structure are responsible for the difficulty in disease management by chemotherapy[i]. Antimalarial drug resistance develops when spontaneously occurring parasite mutants with reduced susceptibility are selected and then transmitted. This is favoured by improper habits in taking medicines. Since most drugs were based on the same targets or lead compounds, drug resistance is emerging and spreading faster than new drugs are being developed. New approaches and strategies for developing drugs and preventing resistance are now coming to light. There is need therefore to track drug resistance to know when to save the current drugs.

Monitoring drug resistance is done mainly by using four basic methods, namely; in vivo tests, in vitro tests, molecular characterization and animal models. Of these, only the first three are done routinely. Additionally, less rigorous methods have been used, such as case reports, case series or passive surveillance. Many discussions have taken place regarding the relative merits of the tests. Careful consideration of the types of information each yields indicates, however, that these are complementary, rather than competing, sources of information about resistance [ii]. This paper revisits the principles of these methods, pinpoints some strengths and challenges and highlights some notions to consider when generating evidence to correlate *in vitro*efficacy and resistance in Cameroon.

Pharmacodynamics and in vivo tests

Pharmacodynamics is the study of the biochemical and physiological effects of drugs on the body or on microorganisms or parasites within or on the body and the mechanisms of drug action and the relationship between drug concentration and effect. Pharmacodynamics is often summarized as the study of what a drug does to the body, as opposed to pharmacokinetics which is the study of what the body does to a drug. An *in vivo* test consists of the treatment of a group of symptomatic and parasitaemic individuals with known doses of drug and the subsequent monitoring of the parasitological and/or clinical response over time. One of the key characteristics of *in vivo* tests is the interplay between host and parasite. Diminished therapeutic efficacy of a drug can be masked by immune clearance of parasites among patients with a high degree of acquired immunity [iii]. Thistest most closely reflects actual clinical or epidemiological situations, offering the best information on the efficacy of antimalarial treatment [iv]. Increased emphasis on clinical response in addition to parasitological response has guided modifications on *in vivo* tests as well as the follow-up periods of 28 days and even 42 days depending on the clearance time of the drug being tested. Failure of complete parasitological clearance, even in situations where recurrence of fever is rare, can be associated with lack of optimal haematological recovery among anaemic patients [v].

Unfortunately, though these methodologies are termed standardized they are in practice not standardized. Major differences in sample size, enrolment criteria, exclusion criteria, length and intensity of follow-up, loss-to-follow-up rates, interpretation and reporting of results are apparent in published papers on *in vivo* trials. These differences are at times so dramatic that it is difficult, if not impossible, to compare results from one study to another with any level of confidence [vi]. The methodology currently being used and promoted, especially in sub-Saharan Africa, is a system that emphasizes clinical response over parasitological response. Besides, measuring drug levels in blood is a must for confounding due to bioavailability.

In vitro tests

In these tests, malaria parasites are exposed to different concentrations of antimalarial drugs in the laboratory. Some methods call for adaptation of parasites to culture first, while others put blood directly from patients into the test system [vii]. The most commonly used methods for antimalarial *in vitro* testing are the *in vitro* micro-test Mark III, the isotopic test and the drug sensitivity assay based on the measurement of histidine-rich protein 2 (HRP2) or *Plasmodium lactate dehydrogenase (pLDH)* in an enzyme-linked immunosorbent assay - ELISA[viii]. In the most frequently used procedure, the micro-technique, parasites obtained from a finger-prick blood sample are exposed in microtitre plates to precisely known quantities of drug and observed for inhibition of maturation into schizonts.

This test more accurately reflects pure anti-malarial drug resistance. Multiple tests can be performed on isolates, several drugs can be assessed simultaneously and experimental drugs can be tested. However, the test has some disadvantages. The correlation of *in vitro* response with clinical response in patients is neither clear nor consistent and the correlation appears to depend on the level of acquired immunity within the population being tested. Prodrugs, such as proguanil, which require host conversion into active metabolites, cannot be tested nor can drugs that require some level of synergism with the host's immune system. Although the adaptation of erythrocytic forms of *P. vivax* to continuous culture has been achieved, non-*falciparum* erythrocytic parasites generally cannot be evaluated *in vitro* [6]. In addition, because parasites must be cultured, differential die-off of parasites can occur. If, for instance, resistant strains are less likely to adapt, the results would be biased towards sensitive responses. Also, because venous blood is typically needed, resistance in the more vulnerable younger age groups is often not studied. Finally, these tests are technologically more demanding and relatively expensive, which makes them potentially more difficult to adapt successfully to routine work in the field. In general, *in vitro* tests can be used to assess patterns of cross-resistance between different drugs, to assess the baseline susceptibility to drugs to be introduced and to temporarily and geographically monitor parasite susceptibility to drugs at country or regional levels.

Molecular techniques

For some drugs (CQ, SP and similar drugs, atovaquone), molecular markers have been identified that confer resistance [ix]. Molecular techniques, such as PCR, gene sequencing and recently real time PCR have been used to identify these markers in blood taken from malaria-infected patients. The dot-blot method has been used to detect point mutations at nucleotide 323 (residue 108) in the *P. falciparum* dihydrofolate reductase (DHFR) gene using allele-specific oligonucleotide probes [x]. The method combines PCR amplification and hybridization of amplified products with radiolabeled allele-specific probes. This technique is specific and sensitive, detects parasitaemia of less than 100 parasites/microlitre of blood and can identify a minority parasite genotype down to 1 % in a mixture.

Serine (Ser) or threonine (Thr) at position 108 of *DHFR* associated with sensitivity to pyrimethamine and asparagine (Asn) associated with resistance have been detected using this method. Restriction Fragment Length Polymorphism (RFLP) has been used frequently to reveal the presence or absence of this mutation [xi]. A restriction site for *Bsr I* from *Bacillus stearothermophilus* (NEB 447) is indicative of the presence of the mutation while its absence is demonstrated through the presence of an *Alu I* (from *E. coli* strain carrying the cloned gene from *Arthrobacter luteus*) restriction site (Figure 1).

These tests are in the process of being developed and validated and offer promising advantages to the methods described above. The advantages include the need for only small amounts of genetic material as opposed to live parasites, independence from host and environmental factors, and the ability to conduct large numbers of tests in a relatively short period of time. The disadvantages include the obvious need for sophisticated equipment and training and the fact that gene mutations that confer antimalarial drug resistance are currently known or debated for only a limited number of drugs (primarily for dihydrofolate reductase inhibitors - pyrimethamine, dihydropteroate synthase inhibitors - sulfadoxine and chloroquine)[xii]. Confirmation of the association between given mutations and actual drug resistance is difficult, especially when resistance involves more than one gene locus and multiple mutations [9]. If these complexities can be resolved, molecular techniques may become an extremely valuable surveillance tool for monitoring the occurrence, spread, or intensification of drug resistance. In a way similar to *in vitro* tests, molecular studies of resistance markers could also provide an early warning system or can target therapeutic efficacy studies.

They can also be useful in monitoring the prevalence of molecular markers in places where a drug has been withdrawn or where a drug combination is in use [xiii]. The development of indices for antimalarial chemotherapy around molecular markers aimed at providing quick answers about the probable efficacy of a drug in a given locality has been explored [xiv] and modified accordingly [xv]. If validated, these indices can provide valuable information for the monitoring of drug resistance based on prevalence of molecular markers.

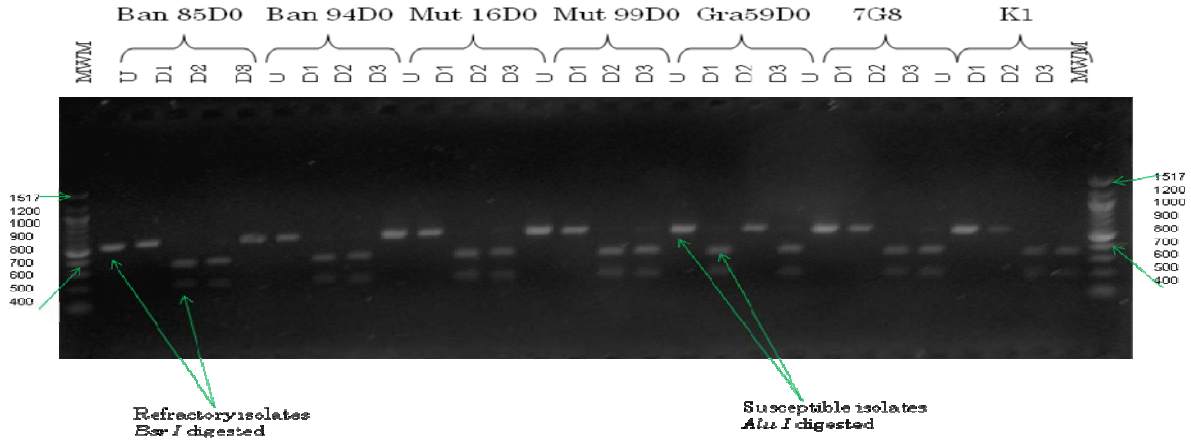

***Figure 1*. Mutant and wild type dhfr of P. falciparum non-respondent to ACTs**

***Legend**: PCR products the dhfr gene (U) digested by Alu I (D1), Bsr I (D2) and Xmn I (D3) restriction enzymes. Bsr I cuts pyrimethamine resistant strain (Asn108) to yield 400 bp and 230bp fragments (Ban 85 D0). Alu I cuts pyrimethamine sensitive strains (Ser108) to yield similar size fragments (Gra 59D0). Xmn I detects pyrimethamine resistant strains (Arg59) digesting the dhfr product to yield fragments of size 420 bp and 210bp respectively. MWM is 100bp ladder Molecular weight markers from New England Biloabls, Samples represent DNA from field isolates from Banso-Bangolan, Mutengene and Garoua, refractory to ACTs. +ve control were laboratory strains 7G8 and K1*

Animal models

This type of test is in essence, an *in vivo* test conducted in a non-human animal model and therefore is influenced by many of the same extrinsic factors as *in vivo* tests. The influence of host immunity is minimized by using lab-reared animals or animal-parasite combinations unlikely to occur in nature, although other host factors would still be present. These tests allow for the testing of parasites which cannot be adapted to *in vitro* environments (provided a suitable animal host is available) and the testing of experimental drugs not yet approved for use in humans. A significant disadvantage is that only parasites that can grow in or are adaptable to non-human primates can be investigated [6].

Additional methods for passively identifying or monitoring antimalarial treatment failures include the use of case reports or case series of spontaneously reported treatment failure. In general, these methods require far less investment of time, money and personnel and can be done on an on-going basis by individual health care centres. Bias is a potential disadvantage in such studies.

In vitro evidence of resistance

For *in vitro* drug sensitivity assay, a given isolate or strain is “drug resistant” when the clinical isolate obtained from a patient (who is not relieved of signs and symptoms, and microscopic examination of blood smears shows malaria parasites, despite administration of the standard therapeutic regimen) has a higher inhibitory concentration (IC50) or minimum inhibitory concentration (MIC) value than the mean values determined for other isolates [xvi,xvii]. Many parallel studies based on the WHO microtest or its modifications have however yielded unexplained discordance between in vitro and in vivo responses to different drugs [xviii]. In some of these studies, the two tests for drug resistance did not show any correlation. The generally poor correlation might have many causes and the explanations might differ for each study [xix]. Some of the possible explanations include the inadequate protocol of the WHO microtest, poor study design based on the WHO 7-day in vivo test, inappropriate drug for the in vitro assay (e.g. amodiaquine instead of monodesethylamodiaquine, arbitrary fixed doses for sulfadoxine-pyrimethamine assay), technical difficulties in obtaining a consistent in vitro response to sulfadoxine, irrelevant choice of study population (e.g. parasitological response in asymptomatic carriers), small sample size, enrolment of patients with recent intake of antimalarial drugs, failure to distinguish between reinfection and recrudescence associated with reappearance of the same isolate as before initial treatment and acquired immunity.

For example, Noedl et al. (2001) [xx] attempted to correlate the therapeutic response to artemisinin combination therapy and in vitro cross-resistance between artemisinin derivatives and mefloquine. The clinical and parasitological response to artemisinin derivatives and the IC50 values for artemisinin were correlated, and the IC50 values for mefloquine and artemisinin were also correlated. The authors concluded that the artemisinin-mefloquine correlation in vitro is reflected in the response to artemisinin treatment. The regimen was not standardized (unspecified artemisinin derivatives, drug(s) used in combination and duration of treatment, delayed administration of mefloquine), and some patients were afebrile throughout treatment and follow-up. Moreover, the success rate of the WHO microtest was low (35/76, 46 %). The *in vitro* responses of isolates from eight patients responding with resistance I (RI) late recrudescence were not specified.

In Yaoundé, Cameroon, Ringwald and collaborators observed about 25% discordance between clinical and parasitological responses to chloroquine and the *in vitro* chloroquine response of the corresponding isolate determined by the radioisotope method[xxi]. Even when patients (aged > 5 years)

with reinfection within 14 days were excluded from the analysis, discordance remained for about 20 % of patients. The study was limited to a 14-day follow-up, as recommended by the WHO protocols existing at that time for areas of intense transmission.

In his book on field application of *in vitro* assays for the sensitivity of human malaria parasites to antimalarial drugs [18], Basco clearly documents the experience of several field researchers who tend to reinforce the followingconclusions:

- *In vitro* assays and clinical studies of therapeutic efficacy do not address the same biological and clinical end-points;
- The thresholds for *in vitro* resistance are fixed arbitrarily, with no reference to predictable clinical and parasitological response.

For these and other reasons cited above, it is difficult to establish a strong correlation between measures determined by the two methods for studying drug resistance. It is more often difficult to explain therapeutic success than therapeutic failure on the basis of *in vitro* assays, possibly because of the major confounding host factor, i.e. acquired immunity.These observations are also valid for current efforts to establish a direct correlation between molecular markers and clinical and parasitological response in patients, especially in terms of predicting the clinical outcome from the DNA of parasite obtained before treatment. *In vitro* drug sensitivity patterns and molecular markers are more closely related from the biological viewpoint, as the phenotype of the parasite “strain’” or population can be considered to be determined by a specific gene that codes for an expression product, which may be an enzyme or transporter protein, that characterizes the observable biological feature. This close relation between the *in vitro* drug sensitivity profile and molecular markers is one of the reasons for maintaining *in vitro* drug sensitivity assays as an important research tool in the field.

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Modelling to Contain Anti-Malaria Drug Resistance

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Background

Malaria is one of the most devastating parasitic diseases of humans that continues to thrive throughout the world and especially in Sub-Saharan Africa, despite the numerous control efforts made so far. It is caused by by protozoan parasites of the genus *Plasmodium,* which is transmitted from one human to another by female *Anopheles* mosquitoes. Five *Plasmodium* species routinely infect humans: *Plasmodium falciparum*, *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium knowlesi* [1]. The high burden of malaria in Africa is also due to *P. falciparum*, the most lethal of the five human malaria parasites, which has co-adapted and co-specialized with *Anopheles gambiae*[2][3], the most effective and widespread vector, making it very difficult to control [4]. Presently, there are about 450 known anopheles species, of which 60 can potentially transmit malaria[2].

There were an estimated 247 million malaria cases among3.3 billion people at risk in 2006, causing nearly a milliondeaths, mostly of children under 5 years. A total of 109 countrieswere endemic for malaria in 2008, 45 within the WHO Africaregion [4]. Annually 25 million pregnancies are potentially at risk [5] with related adverse effects like intrauterine growth retardation (IUGR), low-birth weight (LBW) from prematurity, foetal parasite exposure and congenital infection, infant mortality (IM) linked to preterm-LBW and IUGR-LBW [6]. It is estimated that 75000 to 200000 infants' deaths are associated with malaria in pregnancy [6]. It has been estimated that the economic burden of malaria is extremely high, accounting for a reduction of 1.3% in the annual economic growth rate of countries in which malaria is endemic, and that the consequent long-term impact is a reduction of gross national product (GNP) of more than half [7]. To reduce the extreme burden caused by this disease in Africa, the Millennium Development Goal (MDG) were set out with the aim of reducing malaria by 75% by 2015 from its 2005 baseline level with an average comprehensive malaria control cost of US$ 3.0 billion per year, or around US$ 4.02 billion per African at risk [8] . This does not seemto be enough considering the current impact of malaria. Doolan *et al*. (2009) reported three dominant difficulties in maintaining malaria control[9] :

- Parasite resistance to safe and affordable antimalarials with the spread of unofficial vendors where most of the population go to with elevated risks related to auto-medication (resistance, side-effects, etc);
- The almost complete demise of vector control programs in developing tropical and subtropical countries; and
- The failure to develop a practical vaccine that prevents malaria. Indeed, genetic diversity due to sequence variations in merozoites (1/2) and circumsporozoites (csp) proteins, which is characteristic of malaria parasites, especially *P. falciparum*, has greatly hampered the production of a vaccine.

Between 2004 and 2006, there was a policy change for malaria treatment (adoption of first and second line treatments for artemisinin-based combination therapies).The Cameroon National Malaria Control Programme disseminated this shift in all regions to inform health workers at public and private health facilities. However, much still needs to be done as very little is known about malaria case management in Cameroon [10], as compared to other regions of Africa and given that some health facilities and health workers are still not aware of the policy change. Deficiencies in the practices of both public and private providers compromise the effectiveness and cost-effectiveness of malaria case-management. In 2004, malaria was a major public health problem in Cameroon, and it was considered the first cause of morbidity (NMCP, 2004). However, the morbidity and mortality rates nowadays stand at about 35.88% and 24% respectively. This drop may be attributed to the use of use of Artemisinin-based Combination Therapies (ACTs), Intermittent Preventive Treatment (IPT) in pregnant women, and vector control strategies such as the use of Long Lasting Insecticide Treated Nets (LLITN) and Indoor residual Sprays (IRS) [11].

The discovery on the Thai-Cambodia border of ACTs resistance has raised concerns among the research community especially in Africa. Molecular, systematic and phylogenetic approaches are now being uses among other techniques as predictors of resistance spread. It is generally hypothesized in terms of homology (similarity attributed to descent from a common ancestor) that genes with similar sequences will display similar types of functions or regulations. These have been undertaken in order to identify the nascent point and the emergence of resistance all over the world with contradicting and controversial results that are however promising. This will help to contain the spread of resistance, which can be brought about by population movements and vectors transmission [12][13].

Researchers are now focusing on parasite clearance time after administration of ACTs and some have reported on the fast clearance (short half-life) of artemisinins derivatives in Mali [14]. Others reported that drug resistant strains can also rapidly be cleared by the system[15]. Delay of parasite clearance time has however been noted with artesunate-mefloquine in southern Cambodia [16].

During an infection there is an existing relationship or interaction that takes place between the parasite and the host (human), the two competing actors exerting a pressure on one another. But this requires a dynamic interaction of the genome from the two competitors, reason why it was suggested that the polymorphism of the expression of a set of genes found in humans influences the infection of erythrocytes by *Plasmodium falciparum*[17]. This is the case of the nitric oxide synthase gene that codes for the synthesis of the enzyme nitric oxide synthase (NOS), an antimalarial scavenging reactive oxygen species (ROS) produced by the metabolism of Plasmodium, decreasing NADPH + H and thus preventing infection of new red blood cells and oxidative stress (an imbalance between pro-oxidant and antioxidant in the benefit of anti-oxidant)[18]. Malaria treatment also involves another drug/host interaction in which some human genes are involved: this is pharmacogenetics. One of the genes involved in the pharmacogenetics of antimalarials is the NAT2 gene (N-Acetyl transferase 2), which encodes the enzyme N-Acetyl transferase 2, involved in phase 2 of the biotransformation of antimalarial drugs [19]. The cytochrome CYP gene has also been implicated.

Research on human nutrition has produced very interesting results. Some epidemiological studies in recent years strongly suggest that some minerals (zinc), vitamins (vitamin A) and other trace elements in the diet, may reduce the incidence of such as cardiovascular diseases, malaria, cancer, cataracts and macular degeneration[20]. It has also been suggested that the presence of some elements not yet well characterized, in the milk favours resistance to malaria in Fulanis compared to non-Fulanis[21]. The Fulanis therefore clear parasites faster that the non-Fulanis. In two ethnic groups, Fulani and Mossi, it has also been found that Fulanis had lower risks of contracting malaria infections [22].This was ascribed to the absence of a C allele in the rs2706384 gene of interferon regulator factor 1.

However, Fulani homozygous individuals to C had more chances of contracting malaria. It was demonstrated that some genetic factors in drug metabolism appeared to be substantial contributors to the observed lower efficacy of CoArtem obtained in Cambodia as compared to Tanzania, two different ethnic groups[23].

The decline of parasite clearance time has been attributed to factors such as the age of the individual, the immune system, adherence, toxicity and the area.All these parameters appear to be interrelated and understanding this interrelation necessitates the use of statistical modelling

Models on drug resistance and efficacy

A few models have been fitted using data from different studies to describe the patterns of anti-malarial drug resistance and efficacy. Some of these models have described patterns in drugs utilisations, strategies to delay the progress of drug resistance, the role of anti-malarials in elimination of malaria, and the impact of artemisinin combination therapy and long acting treatment in reducing malaria transmission [24–27].

The impact on the choice of artemisinin combination therapy and the implementation has been described with data from the Thai-Cambodia borders that have shown high drug resistance and also have areas of different endemicity [27].This model has shown that anti-malarial drug resistance spreads faster in low transmission than in high transmission settings. This model has also shown that in low transmission settings, it is treatment failure that is the main cause of drug resistance. Artemisinin has been shown to delay the spread when coverage rates are high and that an exponential inverse effect would be seen in terms of spread of drug resistance if coverage is not adequate. The model predicts the proportion of human population with residual drug levels to be the main determining factor of drug resistance in a setting of high transmission. This model measures rather the spread of drug resistance with the assumption that it already exist.

Hybrid Modelling of three potential benefits of multiple first line treatments (MFTs) have been used to quantify the effectiveness of multiple deployment of artemisinin combination therapies [25]. These models describe the effects reducing the chances of a parasite spreading to other hosts, reducing drug pressure and also reducing parasite fitness to emergence of drug resistance. These models point to the fact that the global emergence of resistance to artemisinin combination therapies is approximately 10 years and that multiple first line therapies has the potential of ensuring a long term efficacy of artemisinin combination therapies, starting with the partner drug.

Other models using malaria data have been used to predict the impact of malaria transmission with the roll out of artemisinin combination therapies and alternative first line treatments in different levels of malaria transmission [24]. This describes malaria transmission in humans and also mosquito populations with respect to some variables that are likely to have an impact on malaria transmission. This model predicts that reduction in the prevalence and incidence of infection associated with a complete switch to artemisinin combination therapies would have more impact in areas where there is a low initial transmission than those with a high transmission rate. This model also shows the advantage of long acting treatments over some currently used artemisinin combination therapies in areas of high transmission. The significance of this model is that, health policy makers need to take into account levels of transmission together with whether a drug is long acting or short acting in recommending an anti-malarial to a community.

There are other researchers that have looked at the epidemiological models for the spread of drug resistance and others on the evolution of multi drug resistance [28][29]. The epidemiological models which basically use the Macdonald-Ross model of malaria transmission, have shown that malaria drug resistance does not spread except a fraction of people that are infected but not treated goes below a thresh hold point. The evolution of multi resistance model explores resistance using the parasite population structure. The premise of the multi-drug resistance model is that the frequency of mutation in the parasite population depends on the proportion of host treated with drugs and the parasite transmission rate. This model has shown that reducing transmission rate is effective in reducing the spread of drug resistance.

Rationale

Anti-malarial drug studies conducted between 1986 and 1992 when monotherapies were still the drugs for treatment of malaria in Cameroon have reported different levels of resistance in different regions. These different levels of resistance, according to the studies depended on what anti-malarial drug was being considered. For example 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 higher in the north (25%) than in the south (2%). Antifolate failures of 12% and 43% *in vivo* and *in vitro* respectively,have been reported in Yaoundé with resistance to aminoquinolines remaining high in the years between 1994 and 2002. Even though in Yaounde, the capital city of Cameroon, amodiaquine and pyronaridine were shown to be efficacious in this same period [30], [31], there were reports in the later years 2005-2010 of declining rates of amodiaquine efficacy or in combination with sulphadoxine pyrimethamine [32], [33].

The replacement of monotherapies by artemisinin combination therapies in the treatment of malaria has greatly improved treatment outcome[34]. Artesunate-amodiaquine and artemether-lumefantrine, are first line and second line treatments of *Plasmodium falciparum* malaria in Cameroon respectively. These choices for first and second line treatment were made without prior data on the efficacy of these drugs. In Cameroon, one of the limitations of new treatment regimens is the frequent problems of supplies, treatment cost and rational use. In the urban areas, there are competing artemisinin combination therapies offered by the private sector and sometime roadside medication vendors[19]. Dihydroartemisinin-piperaquine (Aterkin®) has been introduced commercially and is sold in the drug stores. This led to the decision to evaluate the efficacy and safety of the ACTs-artesunate-amodiaquine dihydroartemisinin-piperaquine and to compare it with artemether-lumefantrine.

The emergence of drug resistance to the very efficacious artemisinin combination therapies in South East Asia [34] remains a great concern to malaria control. There are yet no methods of tracking this resistance. Even though there are artemisinin resistance containment strategies spearheaded by WHO, combating this resistance will require many unknowns to be addressed first [10]. Therefore, modelling the artemisinin combination therapies response to human, ecological and parasite factors has a potential to unravel what might be playing a role in the artemisinin resistance. The time to parasite clearance with respect to these factors could also be helpful in understanding the progress of artemisinin resistance.

The objectives of the study were to :

(i) Assess the efficacy of artesunate-amodiaquine, dihydroartemisinin-piperaquine, in comparison with artemether-lumefantrine for 42 days in children with acute uncomplicated *P. falciparum* malaria, in two different endemic areas;

(ii) Model the effects of drug use, over prescription, auto medication and presence of multiple drugs, ethnicity and endemic region, baseline vital signs, genetic diversity of parasite strains, safety profiles to response to therapy;
(iii) Model the delay in parasite clearance time with respect to drug use, auto medication, ecological region and ethnicity, diversity of parasite strains, safety profiles.

It was hoped that the information gathered would inform policy on the effects of over treatment and efficacy of any 1st line drugs.

Efficacy, safety and non-inferiority of drugs

Artemisinin-based combination therapies (ACTs) continue to gain ground as the most efficacious treatment for uncomplicated *Plasmodium falciparum* malaria. Many studies are looking at the comparative efficacy of the different ACTs in settings where the treatment is most likely to be used[38]. These studies seek to better inform malaria experts and health policy makers on the preferred ACTs or alternatives [38] for the different malaria endemic countries[35- 37].

Results presented herein show high cure rates for the different treatment arms for 14, 28 and 42 follow-up days. These results are consistent with results from studies in other malaria endemic countries in Sub-saharan Africa [35] [37]. The high cure rates of these anti-malaria drugs and the effective use of insecticide treated bed nets could significantly reduce morbidity and mortality in the Cameroonian population (NMCP Cameroon Report 2012). There were similar ACTs cure rates in Mutengene and Garoua. This supports the nationwide implementation of ACTs irrespective of geography and ethnicity and brings an added advantage towards malaria elimination.

The authorities in the Ministry of Health in Cameroon are fighting the illicit sale of medication by road vendors and unauthorized agents. There is still a wide circulation of competing drugs to those recommended by the government for treating malaria. The situation is made worse with the shortages of antimalarials at the different recognized distribution centres [39]. The use of recommended alternative available efficacious and safe drugs is helpful in delaying the antimalarial drug resistance that is beginning to emerge [25].

With the reports of the emergence of drug resistance to the very efficacious artemisinin combination therapies, there is need to monitor the efficacy and safety of these drugs closely. One way to monitor anti-malaria drug resistance in the absence of validated molecular markers and appropriate *in vitro* models is by analysing parasite clearance times [40]. Parasite clearance time curves represented by the proportion of patients that clear parasites with respect to the time from onset of treatment did not show any appreciable delay across the three study drugs. There is still the need for planned monitoring of the *in vivo* parasite clearance times for the different ACTs currently used in Cameroon.

The proportion of patients with a temperature below 37^0C after the 3rd day of first treatment and who remain so for the next 48 hours are similar across the different study drugs. Patients in the ASAQ treatment group and DHP treatment group are cleared of fever by the 3 day faster than their AL treatment group counterparts. This difference however only suggests that patients taking ASAQ and DHP as compared to AL get relieved of the symptom much faster. This does not have any implications on the efficacy of the drugs.

The change in liver function tests: creatinine, alanine aminotransferase (ALAT), aspartate aminotransferase (ASAT) did not show any significant difference between day 0 and day 7 values across the three drug regimens under study. However, there is a significant difference in the day 0 and day7 haemoglobin levels of patients in the AL and DHP. This suggests that patients in the ASAQ treatment group are more likely to achieve convalescence before their counterparts in the AL and DHP treatment group. Though there is need for further research, this result also suggests that iron tablets alongside AL, DHP might be considered in management of malaria patients.

Treatment outcome model

The ability of a patient to fight disease(s) plays a major role in any therapy outcome. In some studies, characteristics like age and sex play great roles. This explains why instead of fitting a simple logistic regression to data, a mixed effect logistic regression is better.

Malaria is associated with anaemia especially during pregnancy [41]. However some artemisinin combination therapies like ASAQ and AL increase haemoglobin levels from day 0 to day 28 at different rates [42]. Even though haemoglobin levels are low in patients with malaria, the sickle cell haemoglobin plays a protective role against malaria [43]. High haemoglobin level is protective against high parasitaemia which also reduces the risk of severe malaria [43].

In our mixed effect model, low haemoglobin levels have been associated with an unfavourable treatment outcome. There is need for association of haemoglobin boosting medication alongside the treatment for a better outcome. Recurrent malaria in children has been associated with poor nutritional status and iron deficiency [20]. Thus the association of iron tablets with artemisinin combination therapies has the potential of a positive outcome and could also curb the recurrence of malaria (especially recrudescence) episodes and could delay resistance to the drugs.

Neutrophils, which play a great role in adverting infections in humans according to our model, influence treatment outcome. High levels have a great chance of killing or suppressing the parasites which eventually lead to patient wellness. All the drugs under study have not shown any association with neutropenia. There is therefore a synergy between high neutrophil levels and treatment in the treatment outcome. Children under the age of 10 who are the cohort under this study were exposed to many infections due to the fact that their immunity is still for the most part innate and therefore, a combined effort by health practitioners needs to be directed to fight other infections common to this age group to save these efficacious drugs.

Alanine aminotransferase which is a regular liver function test in any drug tolerability study has been shown in our study to have a significant effect on treatment outcome. It is indicative of liver injury. Dihydroartemisinin which is one of the drugs in this study has been shown to beassociated with elevated levels of alanine amintransferase. The liver which is responsible for metabolism is very crucial in drug intake to the target cells. A healthy liver will therefore favour a positive outcome of any treatment. Unlike the other patient characteristics, high levels of alanine aminotransferase have been shown in our model (negative parameter estimate) to favour therapy failure.

The drugs which are prescribed for patients also play a great role in therapy outcome. The effect is moderated by the ecological region where this patient is resident.(interaction of site and treatment in our model). In our study, if you are in Garoua, the treatment that might be a good prognosis will be ASAQ. However in Mutengene, DHP seems to have a better prognosis. This result is consistent with studies that show that the recommendation of first line treatments to malaria must not be global but be

on a regional basis [44]. In Cameroon, there is high drug pressure with many drug vendors that are not licensed [45]. Thus, there is a high chance of a malaria patient receiving treatment that does not favour a positive therapy outcome and this might usher in drug resistance to some of the drugs that are in the market. Drug regulation authorities would save the very efficacious drugs so far if they would stringently regulate the type of anti-malarials sold in the different regions of Cameroon.

Parasite clearance time

In this study, we sought to model parasite clearance time in two ecologically different sites, Mutengene and Ngaoundéré, in children aged six months to 10 years following treatment with Arthemeter-Lumefantrine (AL), artesunate-amodiaquine (ASAQ) and Dihydroartemisinin piperaquine (DHP). With the delay in the parasite clearance time that might have contributed to artesunate-mefloquine resistance at the Thai-Cambodia border, ways of circumventing or delaying the onset and spread of resistance have been proposed among which is the implementation of multiple first line therapies [25]. Others have proposed and used models which express best the parasite clearance rate [46]. With such evidence, epidemiological studies will be more accurate and consistent. In this study, the parasite clearance estimator (quadratic or cubic) model was used and added into consideration the initial lag phase that often precede the steady exponential decline of parasite count following antimalarial treatment. We fitted two models (model 1 and 2) and the best fit was obtained for model 1 that showed the lowest Akaike information Criterion (AIC) and Bayesian information criterion (BIC). Although we used a different model (survival model) in our study, the lowest AIC obtained is in agreement with that of the demonstration done by [46] who started fitting their mixed models by looking for a low AIC among their fitted models and preconized their estimator model to be appropriate in estimating parasite clearance time. There are many contributing and interrelated factors to the delay of parasite clearance rate. Integrating these factors in models gives better estimation of each contributing factor to the delay. In our study, we found that age categorized as less than and more than 5 yrs old seemingly does not influence the parasite clearance time. Some studies have suggested age to be an inadequate surrogate marker [47] for immunity especially in low malaria transmission areas. The study suggested an in vitro study for parasite-clearing immunity. This could be controversial as Cameroon is a high malaria transmission area with some protective immunity. In this setting, immunity is age-dependent as was found in Mali [14]by a linear regression model, that parasite artesunate has short half-life which inversely correlated with age. However, a decrease in transmission can explain this apparent discrepancy. We suggest that the model should combine age groups and neutrophils to possibly account for any effect on parasite clearance. Of course we noted that abnormal neutrophils were associated with high persistence parasitaemia. Immunity therefore is a key factor in clearing parasites especially with an adjunct of drug though we noted that the DHP arm was more prone to parasite persistence.

Red blood cells apparently limit the progression of parasitaemia. *Plasmodium falciparum* has the inherent capability of sequestering by the mechanism of adherence in organs making itself absent or almost absent from the peripheral blood circulation. This sequestration limits the presence of red blood cells in the peripheral blood but at the same time circulating parasitized red blood cells (pRBCs) can be targeted by specific antibodies through opsonization, in the presence of drugs[14]. This favours the clearance of the parasite. However, these pRBCs may also account for an underestimation of the real parasite clearance time [26]

It was also noted that fast metabolisers were more prone to have parasite persisting on day one probably owing to the availability of plasma drug [47], which in this study was not evaluated. The trend

was however not significant on day 2 between slow and fast. It is important to note that low level drugs can bring about the onset of resistance.

With respect to site, Garoua showed low probability of parasite persistence, markedly on day 0. Transmission at this moment could be a contributing factor. It has been shown that the severity or persistence of parasitaemia is related to high transmission malaria.

Conclusions

Eventhough at the start of this study, the government policy for first line treatment of *Plasmodium falciparium* malaria was artemether lumefantrine, this research has shown that artesunate amodiaquine and dihydroartemisinin piperaquine are also highly efficacious with cure rates of 95.3% and 96.3% respectively. Better still, this study has shown that these two drugs (artesunate amodiaquine and dihydroartemisin piperaquine) are at least not worse off (with a 10% margin of inferiority) than artemeter lumefantrine in the treatment of *Plasmodium falciparum* malaria in Cameroonian children.

The three drugs in this study are comparatively safe. However those who were treated with dihydroartemisin piperaquine and artemeter lumefantrine show a reduction in haemoglobin levels compared to the levels of the first day of treatment. There was no significant difference when comparing the frequency of the adverse events (mostly vomiting, cough and lack of appetite) that were suspected to be caused by drugs across the different treatments.

Treatment outcome is dependent on the patient's individual characteristics as they come for treatment, the ecological region (site) where the patient is and which drug the patient is given. Some of the patient characteristics that can influence therapy outcome are the neutrophil level and alanine amino transferase before treatment. Low levels of these parameters do not favour a positive outcome. The ecological region (site) where the patient is also influences treatment outcome. However the influence of region on therapy is moderated by the treatment being administered. Garoua which is a savanah sahel region with very long dry seasons do better on treatment outcome with artesunate amodiaquine while Mutengene which is in a forest region with frequent rains, dihydroartemisinin piperaquine favours a better treatment outcome.

Factors that influence the time to parasite clearance are consistent with those that influence outcome. Garoua patients tend to clear their parasite much faster than the Mutengene counterparts. Patients who are treated with dihydroartemisinin piperaquine clear their parasites faster than those in artemeter lumefantrine and artesunate amodiaquine. Low levels of neutrophils and haemoglobin increase the time parasite clearance.

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In Vivo Efficacy Study of Quinine Sulphate in the Treatment of Uncomplicated Plasmodiumfalciparum Malaria in Patients from South Western Cameroon

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Introduction

Resistance of *Plasmodium falciparum* to antimalarial drugs poses a major public health problem to sub-Saharan African countries where malaria is endemic. In addition, the emergence of multidrug-resistant *P. falciparum* especially to easily available and affordable first- and second-line antimalarial therapies in Africa presents new challenges for national drug policies in the region.

Chloroquine and quinine were known to be the two widely prescribed antimalarials in the world [1]. Chloroquine was the mainstay for malaria prevention and treatment in sub-Saharan Africa for over three decades. However, the spread of chloroquine-resistant *P. falciparum* from East through Central to some parts of West Africa resulted in its being dropped as a first line drug choice in the treatment of uncomplicated malaria for Artesunate Combination therapy (ACTs). The very first cases of chloroquine resistance in Cameroon were reported in residents from the town of Limbe in south western Cameroon where resistance was estimated to be 86% in 1986 [2].

Quinine, which was classically reserved for the treatment of severe malaria, was widely used in some African countries as first- or second-line treatment of uncomplicated malaria because of its efficacy and relatively low cost and the increase in resistance of the parasite to chloroquine.

Although Cameroon had an antimalaria drug policy that authorised the use of amodiaquine then as first-line treatment for uncomplicated malaria in Cameroon [3], the choice of first-line antimalaria drugs varied depending on who was prescribing the drug. In a pilot study in the South West region, we observed that quinine sulphate was the most commonly prescribed antimalaria for uncomplicated malaria patients while quinimax (given i.v) infusion was the preferred treatment for severe malaria cases (Achidi *et al*., unpublished data). This treatment regimen had been in use in the South West Region for over a decade (1992-2002) and there were no published studies on the resistance status of *P. falciparum* to this drug in the region.

In response to growing reports by physicians (Sinju, Personal communication) of drug failures following the use of quinine sulphate, we determined the sensitivity and safety of *P. falciparum in vivo* to quinine sulphate in a population of residents of Buea (Fako Division) with the objective of determining the status of *P. falciparum* sensitivity to quinine sulphate thus providing baseline data for future interventions or drug monitoring studies.

Methodology

The study was conducted at the then Buea District Hospital located in Fako Division. Malaria transmission in Buea is perennial with higher transmission during the rainy season (March-November). The main vector for malaria parasite transmission is *Anopheles gambiae* and *P. falciparum* is the predominant malaria parasite accounting for up to 96.8% of malaria infections in the study area [4] The study was carried out during the peak malaria transmission period (May-August, 2002).

Outpatients (8 months to 50 years) of the Buea District Hospital showing symptoms of fever were clinically examined and their axillary temperature recorded. Patients who presented with uncomplicated *P. falciparum* malaria (fever with axillary temperature >37.5 ^{0}C, a parasite density of greater than or equal to 1,000 parasites□l of blood, and any other malaria symptom) and who individually consented or whose guardian/parent granted informed consent to participate in the study were recruited. Patients who had taken any form of malaria treatment within the previous two weeks, had mixed malaria parasite infections, sickle cell disease, severe malaria [5], allergy to quinine sulphate and any evidence of chronic disease or of a concurrent non-malarial febrile illness were excluded from the study.

The World Health Organisation standard 14 days field protocol was followed [5]. The body weights of the recruited patients were recorded; blood films prepared to detect malaria parasitaemia by microscopy using Giemsa stained slides and packed cell volumes were also determined by centrifugation and read on a haematocrit reader. All patients were then treated with quinine sulphate (200mg) sugar coated tablets. The treatment regimen for adults was 30-mg/kg body weight per day given in three daily doses for seven days and in children 15-mg/Kg body weight per day given in three daily doses for seven days.

Study participants reported again on days 1 and 2 for clinical examination and administration of quinine sulphate. Subsequently patients were seen on days 3, 7 and 14 for clinical examination, recording of axillary temperature and those with treatment failure were given artesunate.

Patient outcomes were defined according to the clinical or parasitological response to quinine sulphate therapy. Clinical responses were classified into three groups: early treatment failure (ETF), late treatment failure (LTF) and adequate clinical response (ACR)[5]. Parasitological responses were classified according to four categories: sensitive (clearance of parasites after treatment without subsequent recrudescence), RI (initial clearance followed by recrudescence, early before and late after day 7), RII (reduction of parasitaemia to less than 25% of initial parasitaemia but no clearance) and RIII (no reduction of parasitaemia during follow-up). Anaemia was defined as a PCV < 33 %. The level of significance was set at $P<0.05$.

Results

Seventy-three patients aged between 8 months and 50 years were recruited into the study. Fifty (68.5%) participants were above 15 years of age. Overall the mean (±SD) age of the study participants was 19.56 ± 11.92 (mode = 30 years). The number and ages of male and female study subjects were similar. During follow-up, four study subjects dropped out and 69 patients were followed up till day 14. Baseline clinical and laboratory characteristics of the study subjects are shown in Table 1.

Sixty-nine per cent of study subjects reported a history of previous malaria attack in the prior three months preceding presentation and were treated. Most of these patients (33, 66%) reported taking

over-the-counter antimalarial drugs while 17 (34%) had a hospital-based therapy. We interviewed study subjects on their use of available malaria control measures and observed that only 27.4% of them used one type of control measure at their home. About 15.1% of the subjects had screened houses, 9.6% used insect repellents or insecticides while 2.7% used non-impregnated bed nets.

Clinical and parasitological responses to quinine sulphate

Adverse drug effects occurred in 29 (42%) of the subjects treated with quinine sulphate. The most prevalent adverse effects were tinnitus and dizziness. A total of 65 (94.2%) subjects had adequate clinical response (Table 2) while the remaining four exhibited ETF (1) and LTF (3). Forty (58%) subjects cleared their parasites by day 14.

Haematological response to quinine sulphate therapy

The percentage of subjects who were anaemic before commencement of therapy was 27.4% (7 male children and 13 adult females) and by day 14 the number of anaemic cases had reduced to 17.4% (12/69). Following therapy, haematological recovery was observed in most patients (Fig. 1.)

Discussion

This study demonstrates some evidence of quinine sulphate-resistant falciparum malaria in a study population from South Western Cameroon. Forty-two percent of the patients were unable to clear their parasitaemia (RI-RII) following administration of a standard regimen of quinine sulphate. In a similar study at Kribi (southern Cameroon), a parasitological failure of 20% following quinine sulphate therapy was reported [6]. Other reports from the Philippines showed a full sensitivity of *P. falciparum* to quinine sulphate [1], meanwhile sensitivity rates of 66% and 82% obtained from studies with quinine sulphate and quinine respectively have been reported from Bangladesh [7]. Clinically, quinine sulphate treatment was effective in the study population as we obtained a 94.2% adequate clinical response in treated patients by day 14. A similar result was reported in Burkina Faso where they observed only a few cases of therapeutic failures following quinine sulphate treatment [7]. In contrast, studies in the Philippines [1] and Venezuela [8] recorded a 100% therapeutic efficacy of quinine sulphate.

Previous studies in south-east Asia and in Africa have suggested a relationship between the emergence of chloroquine resistance and reduced sensitivity to quinine [9]. There are many anecdotal reports claiming prevalence rates ranging from 4.3% to 13.2% in the South West Region[4]. Following the development of treatment failures with chloroquine in the 1980s, quinine sulphate then became the drug of choice in the treatment of uncomplicated malaria particularly in this region. Cameroon is located at the crossroads of Central and West Africa. Our report of quinine sulphate-resistant *P. falciparum* in indigenous residents of Cameroon appears to follow a pattern observed in other regions of Africa with chloroquine [10], where chloroquine resistant parasites eventually became widespread throughout the region.

Immunity to malaria increases with age and is strain specific. Previous studies have demonstrated that acquired immunity against *P. falciparum* affects the activity of antimalarials[11]. Furthermore, the efficacy of antimalarial drugs may be augmented by immunity even in the presence of some degree of drug resistance [11]. In our study, the failure rate to quinine sulphate in children was not different when compared with that of adults who are known to have some naturally acquired immunity to malaria. Our observation may be partly due to the fact that most of the children were above 5 years of age. With the

advent of HIV/AIDS, depressed immune systems in adults coupled with malnutrition due to the prevailing difficult economic situation in Cameroon may render adults incapable of controlling infection due to Plasmodium.

As expected, quinine sulphate was well tolerated and accepted by most patients especially as sugar-coated tablets which are known to increase patient compliance [1] were used. However, minor unpleasant side effects of the drug affected 42 % of the subjects and this partly explains why adherence to quinine is difficult even under supervised conditions. In all, no serious side effects and, in particular, no increase in abdominal side effects (nausea, vomiting, anorexia and abdominal pain) or cases of diarrhoea were found in this study.

The use of available malaria control measures by the study population was surprisingly relatively low (27.4%) given the fact that malaria is endemic and perennially transmitted in the study community. Our results suggest the need for sensitisation/education programmes by the malaria control unit on the use of available and effective control measures such as impregnated bed nets.

In conclusion, our study demonstrated that treatment of uncomplicated malaria in the study environment with quinine sulphate resulted in a high adequate clinical response although the parasitological failure was relatively high. It is not clear, however, how widespread this problem is in other parts of the region and nationwide. There is therefore the need to closely monitor the resistance patterns of quinine sulphate in this region and the nation so as to provide useful data to health policy makers.

Recommendations

1. There is need for regular monitoring of resistance to currently used first line antimalarial drug choice in designated sentinel sites to detect possible development of drug resistance.
2. The malaria control programme should prepare and widely disseminate a treatment manual for malaria to all health centres to ensure compliance with treatment guidelines thus reducing the arbitrary use/abuse of antimalarials.
3. Peripheral Laboratories should be equipped with rapid diagnostic test kits to facilitate diagnosis and target therapy of febrile cases. The personnel should also be trained.

***Table 1**. Baseline characteristics of the patients recruited into the quinine sulphate in vivo study*

Characteristics	Values obtained
Number of study subjects	73
Sex ratio (Male: Female)	36:37
Mean age (years)	19.56 ± 11.92 (0.7 – 50)*
Mean body weight (Kg)	49.20 ± 22.30 (8 – 79)*
% who reported antimalarial use prior 3 months	68.5% (50/73)
% who reported use of a quinolone prior 3 months	13.7% (10/73)
Mean number of treatments for malaria prior 3 months	1.31 ± 1.19 (0 – 5)* (median = 1)
Mean duration of presenting symptoms (days)	4.20 ± 3.50 (1 – 21)* (mode = 3)
Mean initial body temperature (ºC)	38.66 ± 1.16 (37.51 –39.13)*
Mean Packed cell volume (%)	35.89 ± 6.32 (18 – 49)*
% of Anaemic subjects (PCV ≤ 33%)	27.4% (20/73)
Geometric mean parasite density (per µL)	15593 (1000 – 133.200)*
Use of a malaria control measure (%)	27.4% (20/73)

**Indicate the range*

Table 2. Clinical and parasitological outcomes of 69 patients post treatment with quinine sulphate for uncomplicated falciparum malaria

Clinical Outcomes (n = 69)	
ACR	94.2% (65)
ETF	1.5% (01)
LTF	4.3% (03)
TREATMENT SUCCESS	94.2% (65)
TREATMENT FAILURE	5.8% (04)
Parasitological Outcomes (n = 69)	
S	58.0% (40)
RI	10.0% (7)
RII	32.0% (22)
RIII	0 % (0)
SENSITIVE	58.0% (40)
RESISTANT	42.0% (29)

ACR (adequate clinical response) = treatment success; ETF (early treatment failure) or LTF (late treatment failure) = treatment failure. RI – RIII = resistant. n = number of subjects

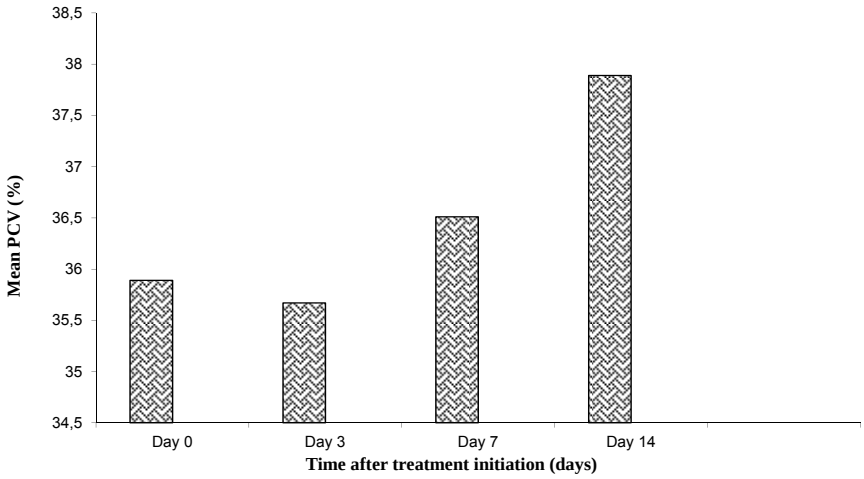

Fig. 5. Mean PCV levels of 69 patients followed-up after treatment with quinine sulphate for uncomplicated malaria.

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Anti-Malaria Resistance Profile and Evolution of Drug Efficacy in Cameroon

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The road to a sustainable malaria control and eradication is very perilous, with numerous impediments such as an environment (vegetation and climate/micro-climate) conducive for disease development and parasite and vector resistance to drugs and insecticide respectively. Various epidemiological studies have so far given a picture of what the state of parasite and vector resistance in Cameroon looks like.

Chloroquino-resistant *Plasmodium falciparum* is now widespread in Africa, and antifolate-resistant *P. falciparum* is emerging in some regions (WHO, 2005). Cameroon, like many other countries, had been forced, following the increasing resistance of chloroquine (Mbacham *et al.,* 2005) (Figure 11), to adopt Amodiaquine and Sulphadoxine-pyremithamine as first- and second- line drugs in 2002 and 2004, respectively. Unfortunately the fall of the cure rates of the latter (Amodiaquine and Sulphadoxine-pyremithamine or Fansidar) were proven to deteriorate (Mbacham et *al.,* 2005) (Figure 12) as monotherapies in five study sites of Cameroon. This led to the adoption of combination therapies in 2004 with Artesunate-Amodiaquine (AS-AQ, Co-Arsucam™) as 1st line drug treatment and in 2006 with Arthemeter-Lumefantrine (AM-LM or AL, Coartem®) as alternative therapy.

Monitoring of drugs resistance is of crucial importance for the anticipation of massive treatment failures and a rapid surge in morbidity rate. Molecular markers for screening and characterization, and evaluation of drug efficacies (assessment of clinical and parasitological responses to drug treatment in malaria infected patients) are available for describing the epidemiology of drug-resistant *Plasmodium falciparum*. The second approach is used to measure the cure rate. Both approaches have been used in Cameroon to determine the distribution of markers of resistance across the country as well as drug efficacy patterns indispensable for an effective treatment policy and for the determination of alternative drugs.

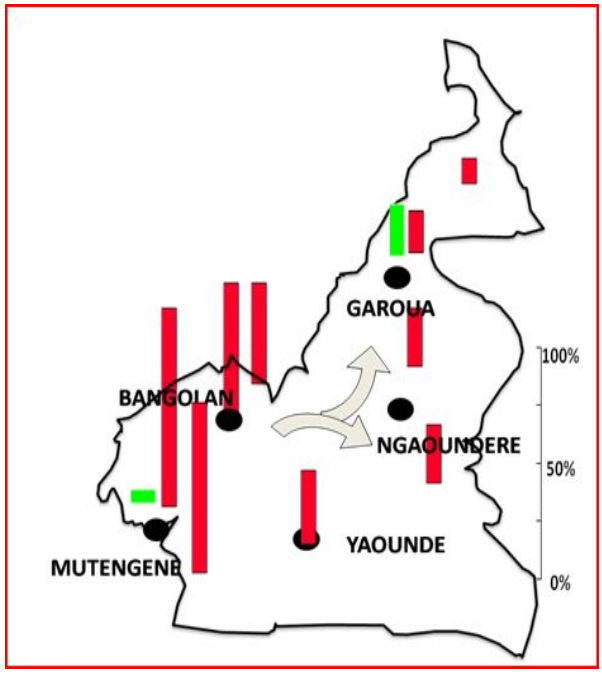

Fig. 1. Antimalarial Drug Resistance in Cameroon (CQ and MQR)

RED BAR (CQR, 2002), **GREEN BAR** (MQR, 1985), CQR : Chloroquine resistance, MQR : Mefloquine resistance (*Mbacham et al*. 2005)

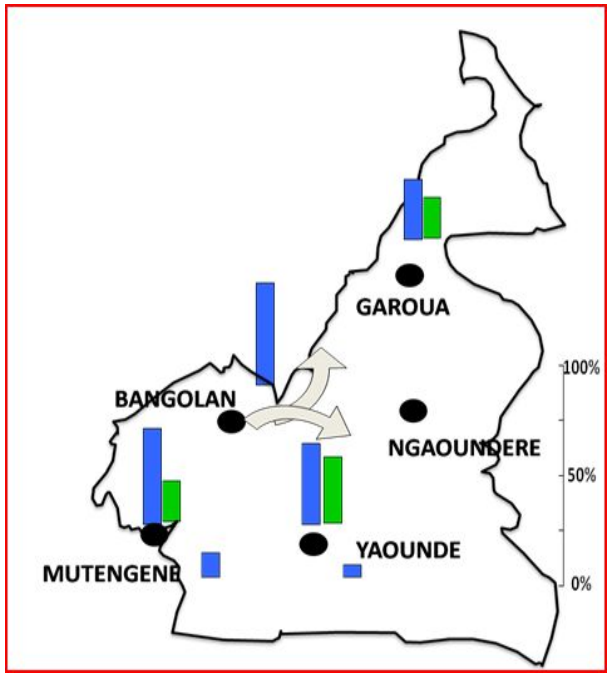

Figure 2. Rising resistance to SP and AQ in Cameroon

***GREEN BAR** (AQR, 2005), **BLUE BAR** (SPR, 2005), AQR: Amodiaquine resistance, SPR: sulfadoxine-pyrimethamine resistance (Mbacham et al. 2005)*

Parasite resistance assessed with molecular markers

Biomarkers such as dyhydropteroate Synthase (*Dhps*), dihydrofolate reductase (*Dhfr*), *Plasmodium falciparum* chloroquine resistance (*Pfcrt*) and *Plasmodium falciparum* multidrug resistance (*Pfmdr1*) are commonly used to assess the efficacy or the degree of resistance of antimalarial drugs. In fact,

occurrence of SNPs (Single Nucleotides Polymorphisms) within these genes (biomarkers) is responsible for the resistance to antimalarial drugs. Sulfadoxine-Pyrimethamine which is still recommended for Intermittent Preventive Treatment in pregnancy (IPTp) and Intermittent Preventive Treatment in infancy (IPTi) in some regions of Africa is a combination of two antifolates compounds that act at two sites of the parasite's folate pathway. It has been used as a substitute to choloroquine. Sulphadoxine inhibits dihydropteroate synthase (*dhps*) gene, whereas Pyremithamine inhibits dihydrofolate reductase (*dhfr*) gene in the folate pathway of the parasite. This combination acts in synergy. Resistance to this therapy is due to the accumulation of mutations (SNPs) in codons 108, 164, 59 and 51 (Plowe *et al.* 1996). These mutations alter the configuration of the active site and consequently reduce the affinity for active compound. *Dhfr* mutations can be specific or interact with *dhps* codon 437 and initiate resistance to SP.

A plethora of studies have considerably reported on resistance to drugs such as chloroquine (CQ), Quinoline, Mefloquine (MQ), Artemisinins, Amodiaquine (AQ), Lumefantrine (L), Quinine etc. associated with SNPs in these markers. It was noticed that *Pfcrt* 76 T is highly associated with CQ resistance (Lopes *et al.*, 2002) and *Pfmdr1* 86N to MQ and lumefantrine (Sisowath *et al.* 2005). Djimde *et al.* (2001) reported *Pfmdr1* 86 Y as an important modular for CQ resistance; the mutation does not itself confer resistance to CQ. Other mutations, namely *Pfmdr1* 184 F and 1246 Y, have also been reported to be highly associated with increase resistance to CQ (Foote *et al.* 1990).

In Cameroon, the mutations profile of these markers, notably *Pfcrt* and *Pfmdr1* has been done in three sites namely Garoua, Yaounde and Limbe (Mbacham *et al*.,unpublished data). Results showed that mutations in *Pfmdr1* 86 Y/ 184 F and *Pfcrt* 76 T predominated in the three sites with the exception of *Pfcrt* 76 T and *Pfmdr1* 86 Y which were less marked in Garoua. Amodiaquine resistance (AQR) was also less marked in the three study sites and the *Pfmdr1* 1246 Y mutation was very low (Figure 13). The following observations were made:

- The triple IRN mutation could not be conveniently associated with treatment failure;
- Presence of Pfcrt 76T in parasite was not associated with AQSP or AQ failure;
- Presence Pfmdr-1 86Y was not associated with AQSP or AQ failure;
- Presence of Pfcrt 76T plus Pfmdr 1 86Y (TY) in parasite was not associated with AQSP or AQ failure;
- Presence Pfmdr 1 86Y plus Pfmdr 1 184F plus Pfmdr 1 1246Y (YFY haplotype) mutations in parasite was not associated with AQ or AQSP failure.

The afore-mentioned study also established that there was a positive relationship between SGK resistant allele (Serine-Glycine-Lysine) and SP clinical failure. Besides, it was noticed that SGK and AGK alleles were highly represented in Limbe, which seems to predispose Limbe to SP clinical failure. It should be noted that SGK resistance is associated with *dhps* biomarker, with serine, glycine and lysine mutations at codons 436, 437 and 540 of this gene, respectively.

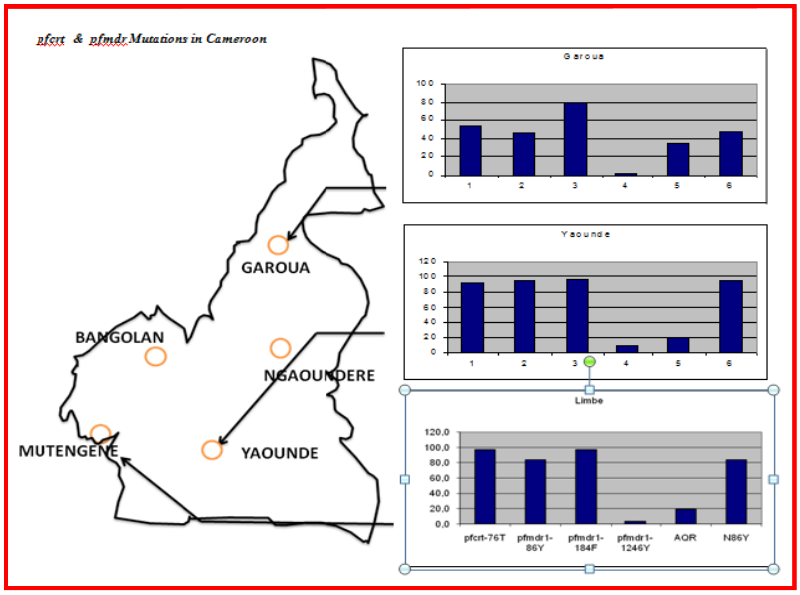

***Figure 3**. Pfcrt and Pfmdr1 Mutations profile in Cameroon (Mbacham et al., Unpublished data)*

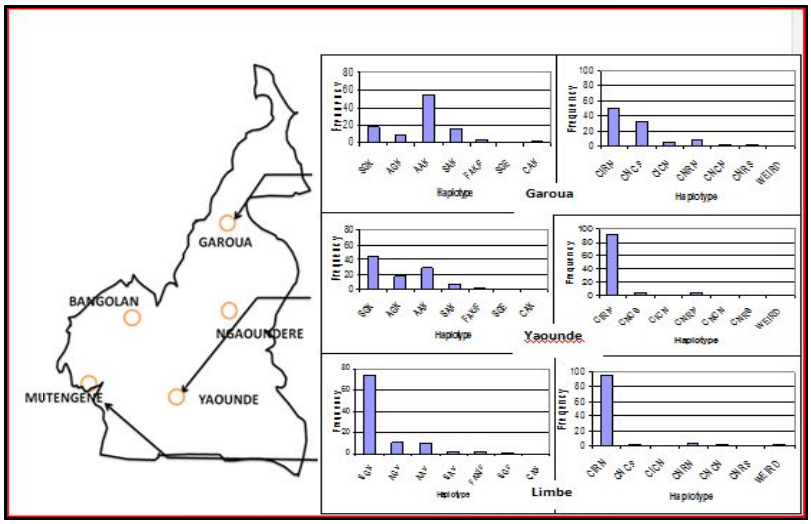

***Figure 4**. Dhps and dhfr mutations profiles in Cameroon (Mbacham et al., unpublished data)*

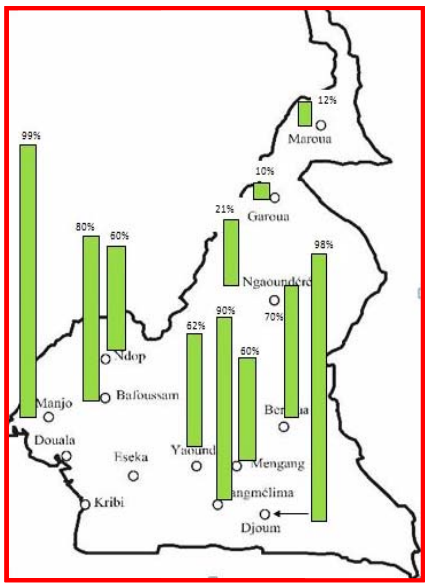

***Figure 5**. Geographical distribution of Dhfr triple mutant alleles IRN in 11 localities of Cameroon*

Mutation rate: Manjo (Littoral) > Djoum (South)> Sangmelima (South)> Bafoussam (West) > Bertoua (East) > Yaoundé (Centre) > Mengang (South) = Ndop (North West) > Ngaoundéré > Maroua > Garoua. IRN: I represent isoleucine with mutation at position 51; R, Arginine – 59; and N, Asparagine – 108 (Ile – 51/Arg – 59/Asn – 108).

Source : Adapted from Tahar and Basco (2006).

As concerns *dhfr* gene mutation, the triple mutant allele IRN (Ile-51, Arg-59, Asn-108) was found to be predominant in the Centre, South, Littoral and West regions with an overall percentage of 62.2% in the Country (Figure 5). The Northern part of Cameroon (Ngaoundéré, Maroua and Garoua) had the lowest rate of mutation for *dhfr*, followed by the East region (Bertoua, 42% in 1999).

Wild-type was mainly marked in Northern parts (Ngaoundere, Garoua (2001), Maroua) (Tahar and Basco. 2006) (Figure 6).

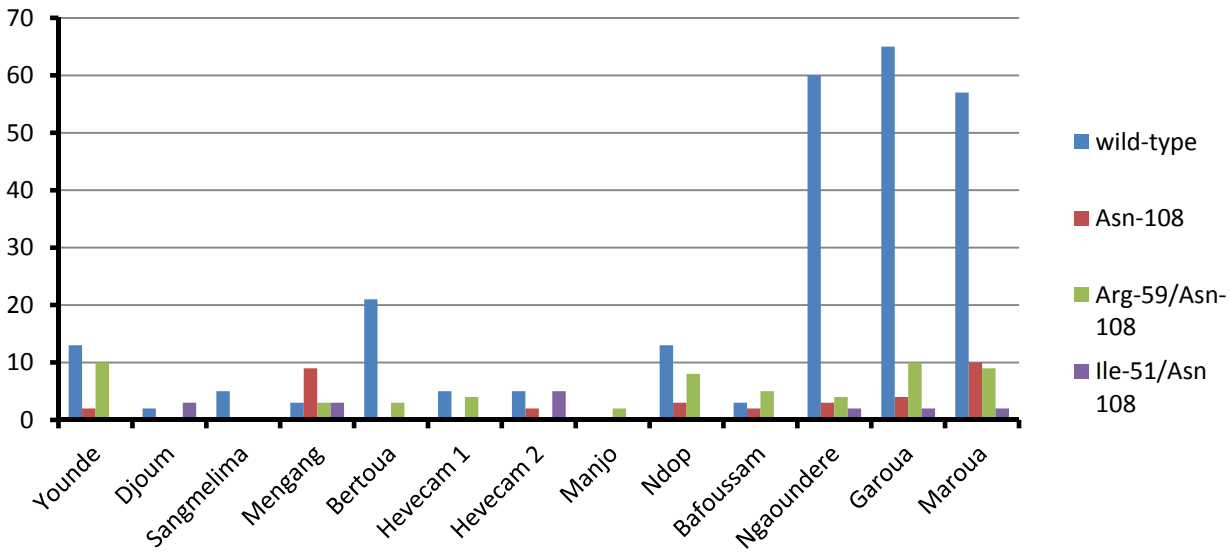

***Figure 6* : Distribution (%) of Dhfr gene wild types and mutant alleles in 13 areas of Cameroon**

Legend : Asn: Asparagine, Arg: Arginine, Ile: Isoleucine.

Source : Adapted from Tahar and Basco. (2006)

Compared to the triple mutation (I51/R59/ N108) that has no great impact on the sensitivity of chlorproguanil-dapsone (CD), another potent antifolate combination (Watkins *et al.* 1997, Nzila-Mounda *et al.* 1998) withstrong resistance to CD and to SP, has also been ascribed to a quadruple mutant form (N108/I51/R59/I164L) of the *dhfr* gene as reported in Asia and Latin American countries (Wichmann *et al.* 2003). Plowe *et al.* (1998) reported that in this fourth mutation, the enzyme is about a 1,000 fold less sensitive to pyremithamine. The presence of this mutation (quadruple mutant) in *Plasmodium falciparum* isolates imported from Central, West, South, East-Africa and Madagascar, to Europe, has been assessed and none was found among these isolates (Wichmann *et al.* 2003). In Cameroon, a study realized on this aspect showed no association between the quadruple mutant genotypes and SP or AQSP failures and besides, if any, the frequencies of quadruple genotypes were very low (Mbacham *et al*., Unpublished data). Nevertheless, continuous surveillance is necessary (Wichmann *et al*. 2003) as the dispersal of resistance patterns across regions could also be due to population movements and therefore parasite migration patterns as suggested by Pearce *et al.* (2009).

To investigate the evolutionary origins of *dhps* mutations, Pearce *et al.* (2009) examined diversity at microsatellites markers flanking the gene and characterized five major lineages with the geographical

distribution of *dhps* resistant alleles mutations: SGK (Serine-Glycine-Lysine), AGK (Alanine-Glycine-Lysine), and SGE (Serine-Glycine-Glutamate) (Figure 7), wild-type alleles AAK (Alanine-Alanine-Lysine) and SAK (Serine-Alanine-Lysine), (Figure 8B) in various regions of Africa. The authors suggested that from the frequencies of resistance lineages expressed in the pie chart map, there has been dispersal throughout West and Central Africa from their original foci, with Cameroon at the confluence of West, Central, and South West African gene pools (Figure 7). In Cameroon it was found that the resistant haplotypes AGK/SGK 3 was mostly predominant compared to other geographical regions with mainly AGK/SGK 1(Central and South East African sites) and AGK/SGK 2 (West African sites) (Figure 7). These results may express a likely difference in antifolate sensitivity as underlined by the authors.

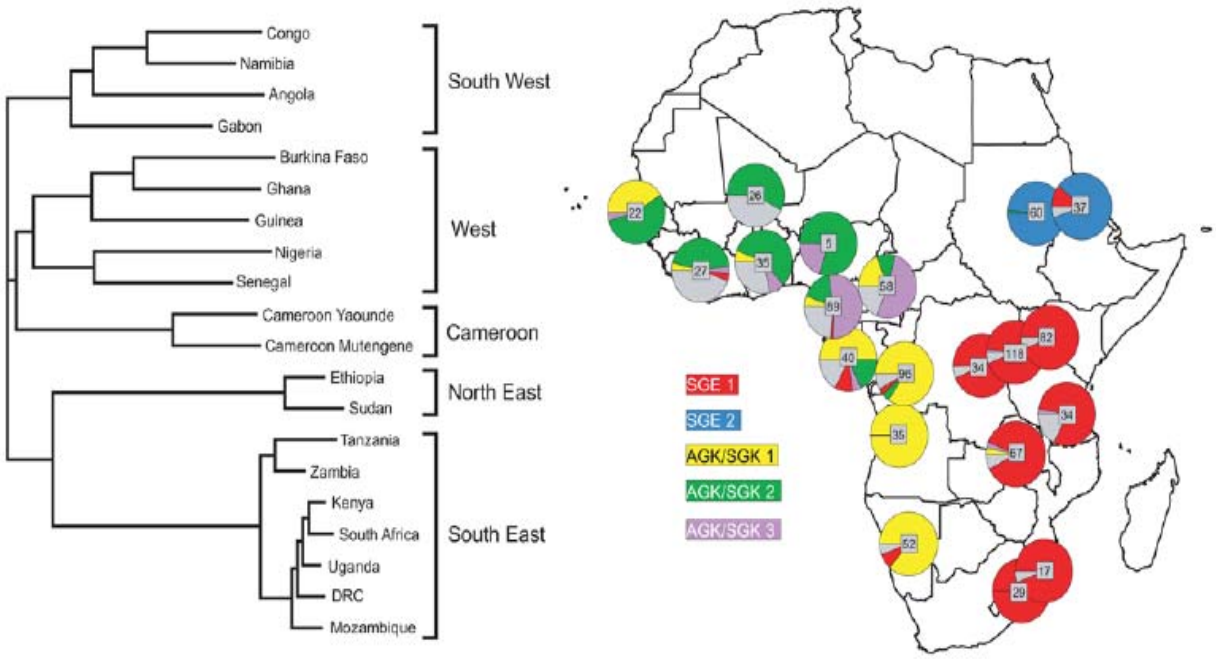

Figure 7. The African distribution of dhps resistance lineages (Pearce et al. 2009).

In Cameroon, AGK /SGK alleles were mostly marked in Mutengene, followed by Yaounde and Garoua (Figure 8) whereas wild-type alleles (SAK and AAK) were mostly marked in Garoua, Yaounde and Mutengene respectively. The result with SGKcorroborates that of Mbacham *et al*. (2010)(Figure 8).

Various studies have suggested that triple *dhfr* mutations with or without additional mutations in *dhps* gene, are associated with clinical resistance to SP in Africa (Basco *et al.* 2000; Nzila *et al.* 2000; Mockenhaupt *et al.* 2005; Mbacham *et al*. unpublished data; Pearce *et al*. 2009). This may have great impact as SP is still recommended in most African regions as IPTp (Intermittent preventive treatment for pregnant women) and/or IPTi (Intermittent Preventive Treatment for infants).

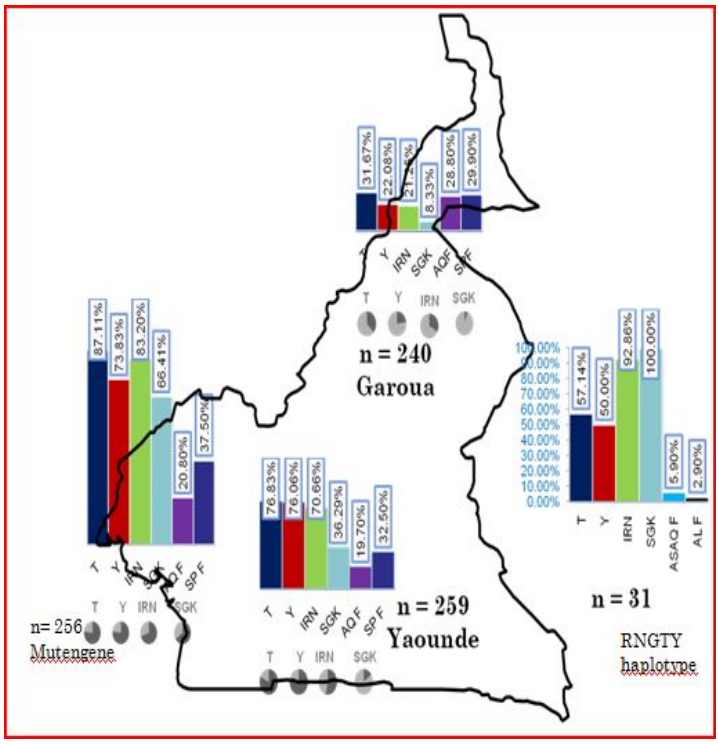

Figure 8. Antimalarial drug resistance markers

T represents the threonine (T) mutation on codon 76 (76T) of the Pfcrt gene. Y represents tyrosine (Y) mutation at codon 86 (86Y) of the Pfmdr1 gene as putative markers for Amodiaquine failure (AQF (D28)). Sulphadoxine-pyrimethamine failure (SPF (D28)) whose molecular markers, IRN represents the isoleucine, arginine and asparagines mutations at codons 51, 59 and 108 (IRN) of the dhfr gene and SGK represents the serine, glycine and lysine mutations at codon 436, 437 and 540 of the dhps gene. (Source: Mbacham et al., 2010)

Parasite resistance evaluated by clinical assessment of drug efficacy

As previously mentioned, drug efficacy can be assessed with principal outcome as ACPR (Acute Clinical and Parasitological Response) on D28 or D14 as recommended by the WHO. Other variables include the ETF (Early Treatment Failure), LCF (Late Clinical Failure), LPF (late parasitological failure) and LTF (Late Treatment Failure). On these grounds, various studies have reported on the efficacy of both non-Artemisinins and Artemisinins based combination therapies for malaria treatment.

As part of the surveillance programme for the therapeutic efficacy of the first line (CQ and AQ) and second line (SP) drugs for the management of uncomplicated *Plasmodium faciparum* infections, Basco *et al*. (2006) conducted non randomized studies in symptomatic children aged less than 10 years according to the WHO protocol (14 day follow up period) at 12 sentinel sites in Cameroon between 1999 and 2004. A total of 1,407 patients were included in the studies. Of these patients, 460, 444 and 503 were assigned to CQ, AQ or SP treatment groups respectively. Chloroquine resulted in high overall failure rates (ETF + LCF + LPF, 48.6%) (Figure 9). Chloroquine was ineffective in Centre, South, East and West regions of the country. Though the number of sites was limited, there seemed to be a gradient with decreasing failures rates towards the Sahelian North (from 38% in Ngaoundéré to 20% in Garoua and 16% in Maroua). Amodiaquine was highly effective in all study sites (Figure 10). The overall cure rate (i.e. ACPR) was 92.7% on Day 14. Most of the failure rates were due to LPF. In Yaoundé, AQ efficacy was evaluated in 1999 and 2003. There was no indication of change in the efficacy of the drug between these two time periods. Compared with AQ, SP was less effective, with

47 of 475 (9.9%) patients failing to respond to the treatment. Close to half of these patients (20 of 47 failures, 43%) required an alternative treatment on or before day 3 due to ETF. SP efficacy was evaluated in Yaoundé in 1999 and 2003, and in Hévécam in 2001 (no failure) and 2004 (11.1% failure) (Figure 11).

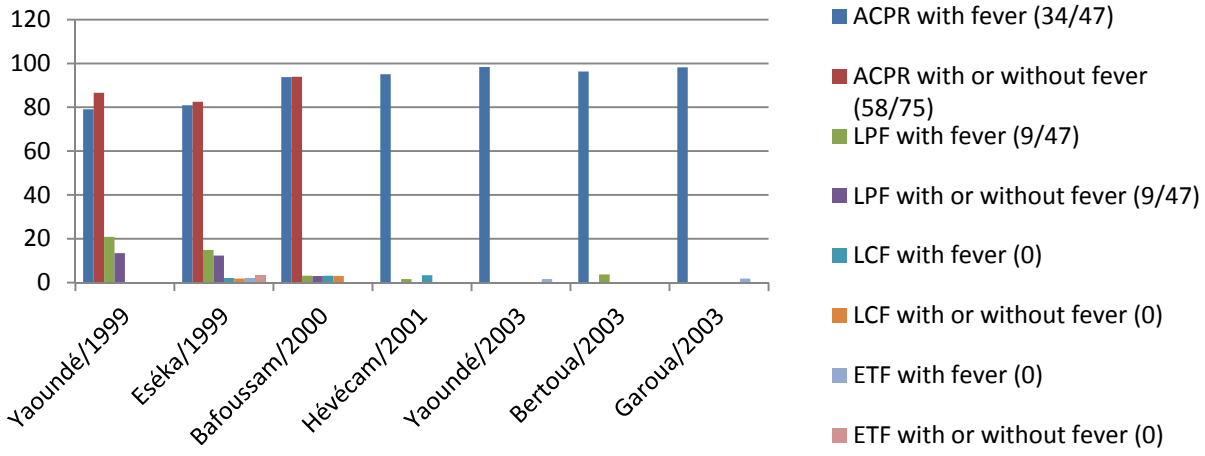

Figure 9: Therapeutic efficacy of Amodiaquine in Cameroonian children, 1999-2003 (Basco et al. 2006)

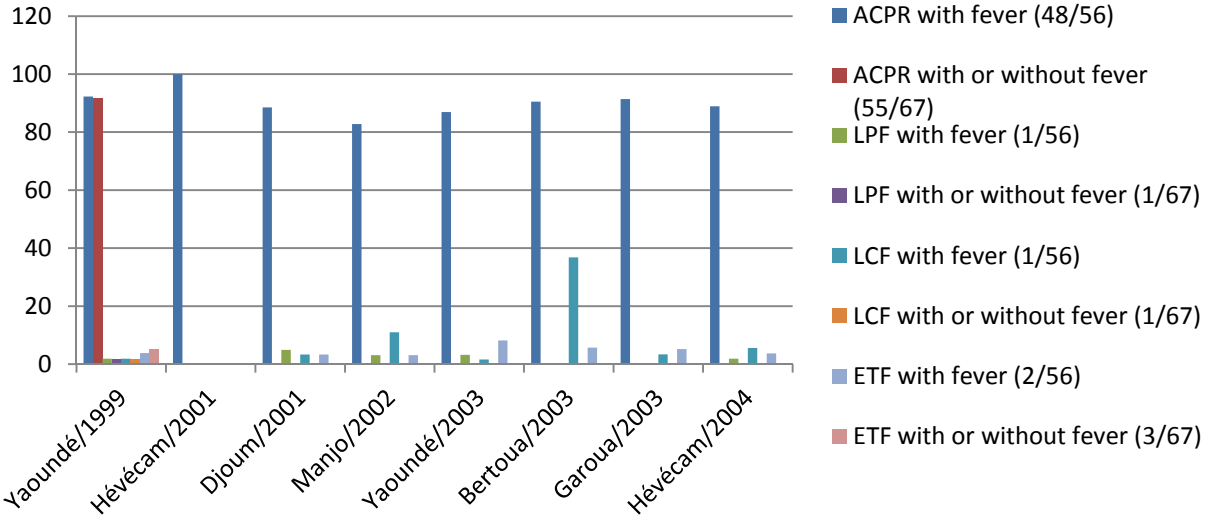

Figure 10 : Therapeutic efficacy of sulfadoxine-pyrimethamine in Cameroonian children, 1999-2004
(Source: Basco et al. 2006)

This finding has also been reported by Whegang *et al.* (2010) in the comparison of AQ, SP and AQ-SP efficacy on D14 in three sites of Cameroon, namely Yaounde, Bertoua and Garoua. The overall cure rate (CR) of AQ-SP on D14 (PCR uncorrected) was 93% for Yaoundé/Bertoua/Garoua (Figures10 and 11). AQ-SP CR was however, not statistically different from AQ, and SP was less effective than AQ-SP, with an overall CR of 87%.

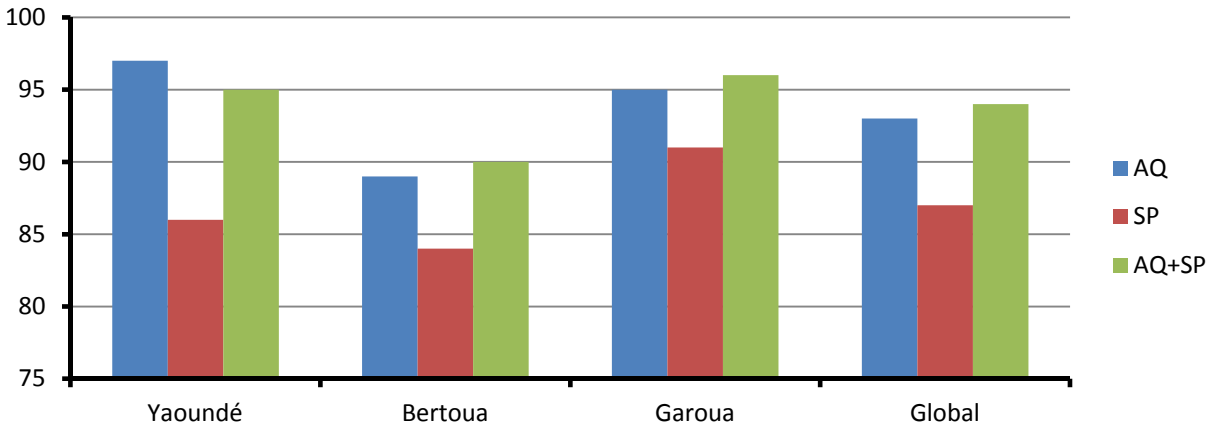

***Figure 11**. Efficacy of AQ, SP and AQ-SP in Cameroon on D14 (Whegang et al. 2010)*

As reported by the latter author, AQ monotherapy is still effective in Cameroon but should be protected with artesunate (or SP) to delay the emergence of resistance. The current trend in Africa is to reserve SP for the intermittent preventive treatment in pregnant women (Newman *et al.* 2003). During the transition period before the implementation of the new drug policy based on ACT, AQ-SP combination has been proposed by some malaria experts to be an effective, alternative non-ACT combination (WHO, 2001). The results showed that AQ-SP combination was more effective than AQ and SP monotherapies. AQ-SP was as effective as AS-AQ combination, as already shown in a meta-analysis in Africa (Obonyo *et al.* 2007). The advantages of AQ-SP combination include its high efficacy, good tolerance, suitability for young children, and immediate availability of both drugs in many areas in Africa and relatively low price of the generic drugs (Whegang *et al.* 2010). Therefore, this non-ACT would have been a useful alternative during the transition period towards the full implementation of ACT to mutually protect AQ and SP in African countries where these two drugs are still effective. Nevertheless, Mbacham *et al.* (2010), as they were assessing the efficacy of AQ, SP and AQ-SP for the treatment of uncomplicated malaria on D28 found that these drugs were highly associated with in vivo failures and high prevalence of resistant genes *pfcrt*, pfmdr I, *dhfr*, *and dhps*. Though a relatively high efficacy was observed as shown in Figure 12, this seems not to be enough according to WHO (2005). The latter, recommends that an antimalarial drug should be as efficacious as 95%. This finding reinforced the position of the Cameroon government in the recommendation for changing its first line treatment from monotherapies to ACTs.

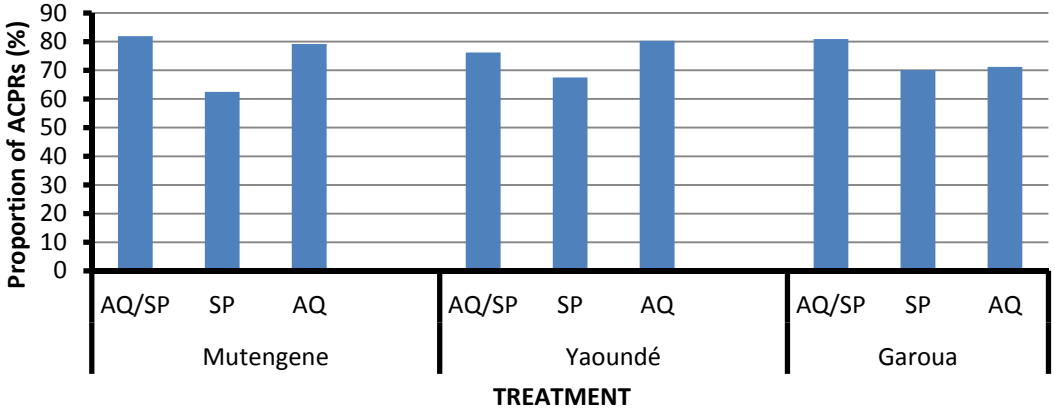

***Figure 12**. Adequate clinical and parasitological responses (ACPRs) in the 28 day trial for non-artemisinin combination therapy (AQ-SP), AQ, and SP after PCR correction in three zones of Cameroon (Source: Mbacham et al. 2010).*

It should be noted that, the recommended follow-up duration for malaria treatment in clinical trials is ≥ 28 days in areas of high as well as low to moderate transmission. In effect, a significant proportion of treatment failures do not appear until day after D14. Thus, shorter observations periods lead to a considerable overestimation of the efficacy of the tested drug (WHO M*alaria treatment guidelines, 2006*).

The combination treatments mostly and commonly recommended for Africa are non-Artemisinins combination such as AQ-SP, and ACTs such as AS-AQ, AS-SP, AM-LM. Other forms of ACTs include AS-MQ, DH-PP, AS-CD, Artesunate pyronaridine, Artesunate-atovaquone-proguanil. The study carried out Whegang *et al.* (2010) focused on the efficacy of all the above-cited antimalarials. The aim was provide a rational basis to consolidate the implementation of ACTs throughout Cameroon and also to provide a baseline data for possible adjustments and modifications in the national antimalarial drug policy in the future. The results obtained suggested AS-AQ to be as effective as AS-SP, AM-LM, AS-MQ, AS-CD and DH-PP. AM-LM appeared to be the most effective followed by DH-PP (Figure 13).

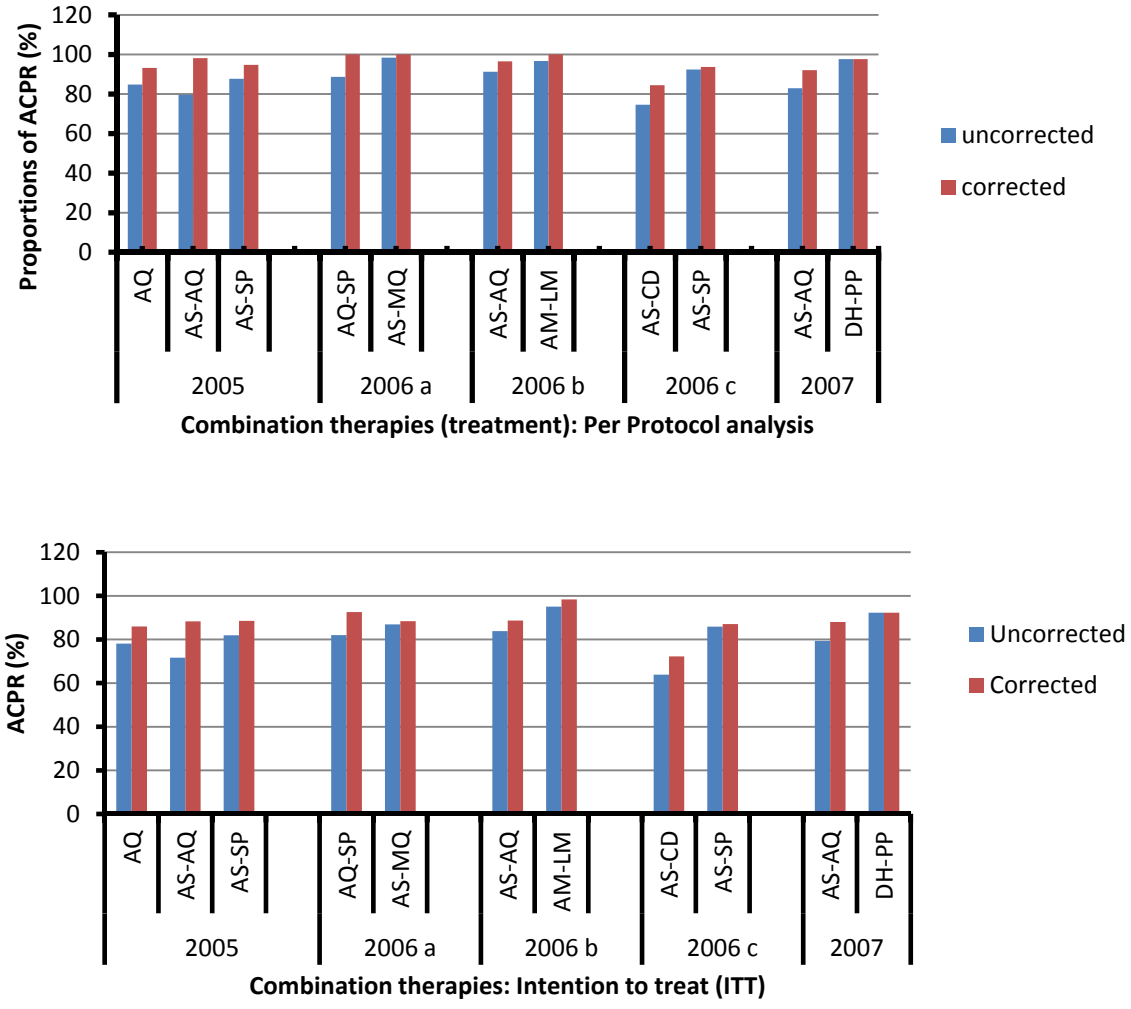

Figure 13. *Adequate clinical and parasitological responses (ACPRs) in the 28 day trial for combination therapies before and after PCR correction from 2005-2007 in Yaoundé Cameroon (Per Protocol and intention to treat ITT) (Whegang et al., 2010).*

For an alternative drug regimen, Whegang *et al.* (2010) observed that, AM-LM has potential advantages over other forms of ACT although it requires six doses, rather than the three doses given for other artemisinin-based combinations. She recommended further studies to evaluate the clinical efficacy and tolerance of these combinations in different epidemiological situations. It is worthnoting that the evaluation of drug efficacy should not only be assessed as the ACPR on D28 or D14 as recommended by WHO guidelines but also in the ease of administration, which is a key determinant of compliance, thus efficacy with shorter courses and fewer tablets being preferred over the current minimum of three days and multiple tablets a day for most forms of ACTs. As recommended by WHO experts, the ideal anti-malarial drug should have an efficacy of at least 95% as measured over 28 days of follow-up. They also recommend that re-infection in that period should be restricted to a few pills administered as a single dose and should have a short treatment duration (Mendis, 2005). A fixed-dose ACT (FDC) would be able to improve compliance of the treatment and reduce the cost of malarial treatment in endemic countries. Thus to improve the existing ACT, (AS) was combined with sulpha-methoxypyrazine-pyremithamine (SMP/Co-Arinate®) in a co-blister, taken once daily (two tablets simultaneously) for over three days. This combination is available as a prescription drug in many African countries. As reported by other studies, the dosing interval of Co-Arinate could be reduced to 12 hours enabling a 24-hour treatment (Adam *et al.* 2006; Penali *et al.* 2008). In 2009, Sagara *et al.* (2009) investigated the effectiveness of this dosage regimen compared with the same treatment given over 48 hours (dose interval of 24 hours) using as standard therapy Coartem® (AL FDC, the six dose regimen). Four African countries (Cameroon, Mali, Sudan and Rwanda) were included in the trail. As for Cameroon, the efficacy measured by the ACPR on D28 (after PCR correction) per protocol analysis is given in Figure 14. AS-SMP three days or AS-SMP 24 hours are as efficacious as Arthemether-Lumefantrine and well tolerated. However, slight adverse effects such as vomiting and diarrhoea were observed in the AS-SMP 24-hours group. This finding also provides the basis for implementation of alternative ACTs for policy change as in the case of others ACTs (Whegang *et al.*, 2010).

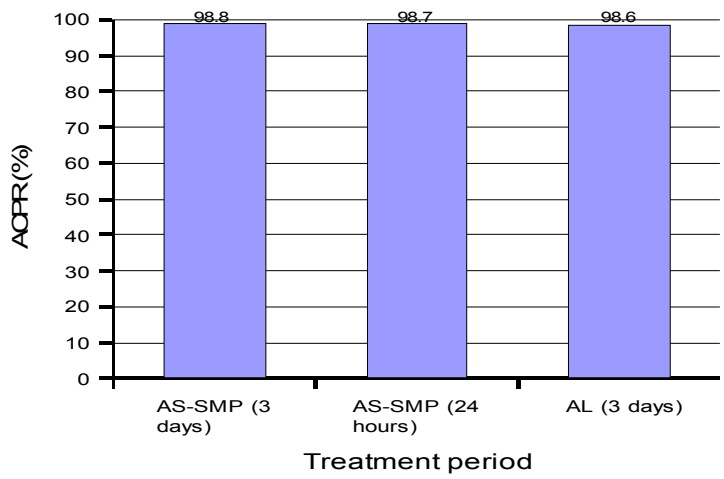

Figure 14*. Evaluation of ACTs efficacy on different treatment periods with AL as standard*
AS: artesunate, SMP: sulphamethoxypyrazine-pyremithamine, AL: Arthemether-Lumefantrine
(Source: Sagara et al. 2009)

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Challenges of ACT Subsidy : The Cameroon Case File

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Fobang foundation,FFISH consultancy group, ***University of Yaoundé 1 - Study done by the Fobang Foundation, sponsored by the DALBERG group through the Malaria Consortium*

Cameroon revised its treatment policies for the treatment of uncomplicated malaria in 2004 and moved from monotherapies (such as chloroquine now subject to resistance) to Artemisinin based combination therapy (ACT). About three years passed before the first massive deployment of ACTs through the public sector outlets was recorded. In order to increase access to malaria drugs, Cameron received and used subsidy funds of the GFATM, Round 5 funds, to subsidize the purchase and distribution of ACTs in private and public sectors with different mark-up rates.
This study was carried out in North West, South West and Centre Regions in order to:

- Verify whether the subsidy passed on through the distribution channels reached the point of sale;
- Describe the current anti-malarial market and the GFATM project and annual ACT import quantities;
- Establish data on actual prices paid;
- Describe the ACT pricing and availability data.

Methodology

The study was carried out from October to December 2007 and the methodology consisted of a cross-sectional survey in three of the ten regions of Cameroon, namely, Centre region (excessive drug pressure), North West region (self-reliant development efforts) and Southwest region (trade influence with neighbouring Nigeria).

Structured questionnaires were used to collect data from pharmacies and wholesale distribution units. For each serving public hospital pro-pharmacy in the main urban centres of Yaounde, Bamenda and Buea, the pro-pharmacy and 4 outlets around it were studied. Three outlets in addition to the pro-pharmacy per serving hospital or clinic were studied for each small city. In each rural setting district health facility, the pro-pharmacy was matched to one drug outlet. A mystery shopper survey to determine actual prices paid by consumers was done. Data was also collected from importers/wholesalers/central medical store level to determine uptake of ACT through the private sector.

National drug procurement system

Cameroon's population is about 18,579,499 and there are only 280 functional pharmacies, giving a ratio of 66,356 persons per pharmacy. There are no chains of pharmacies. CENAME, Central warehouse of the Ministry of Health, is the only supplier of subsidized ACT registered with AS-AQ and Art-Lum references. The distribution of these products begins from CENAME and radiates towards the

regions and subsequently to the health facilities and then the patients. There is a parallel drug procurement scheme either - The Special Drug Fund operating in the North West and South West regions or the black market drugs from which all providers including private faith-based or illicit street vendors procure drugs (Figure 1).

The above diagrams (Figure 1 and 2) illustrate an ideal drug distribution network in Cameroon and present the actual situation with a parallel drug distribution network. The unidentified network functions well whenever the government distribution network is unable to satisfy the needs of the health facilities and compete favourably.

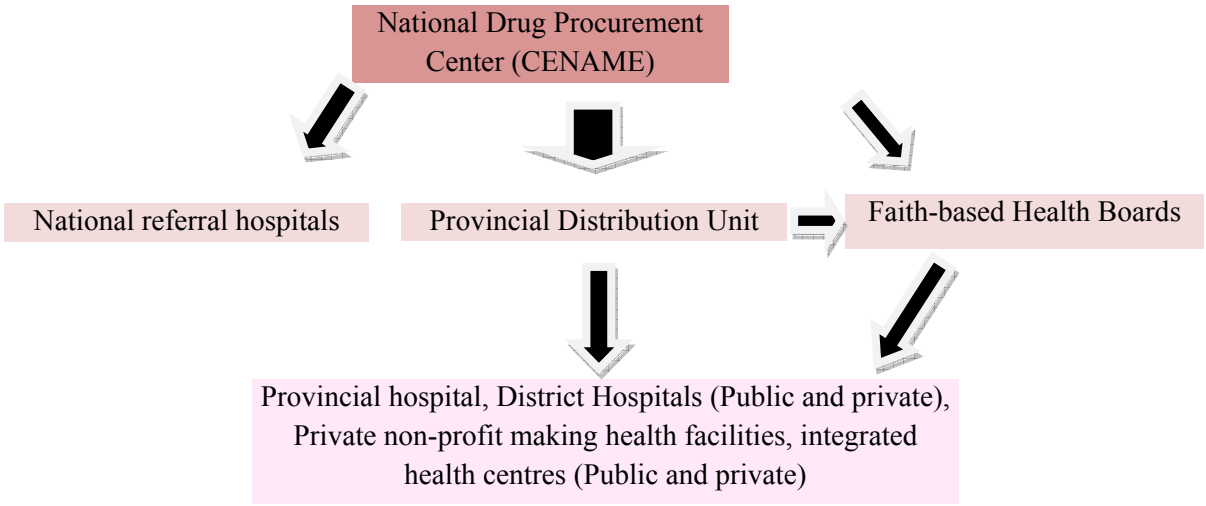


***Figure 1** : Deal flow chart of drug procurement in the health system in Cameroon*

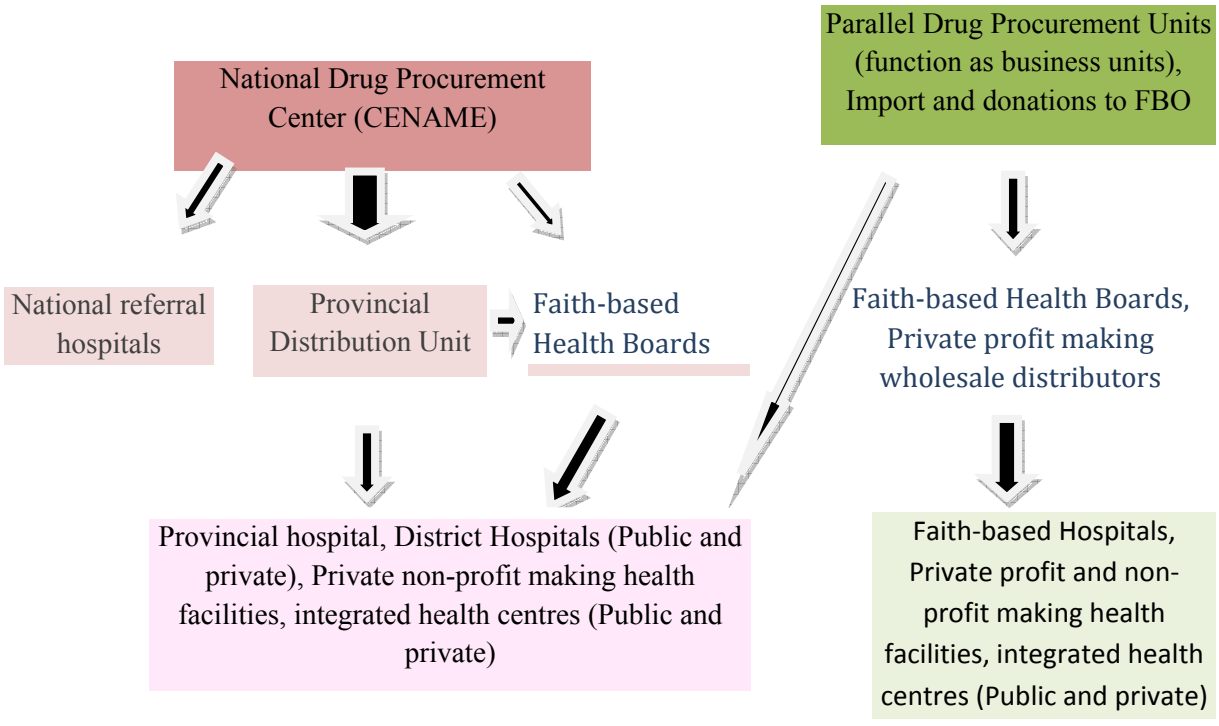


***Figure 2**. Actual flow chart of drug procurement in the health system in Cameroon*

Current antimalarial market

Within the GFATM programme, the companies supplying the ACTs to CENAME were as follows: CIPLA (70% of AS-AQ (co-blister), SANOFI-Aventis (30% of AS-AQ (FDC)) and MISSION Pharma (100% of Art-Lum). The Ministry of Health allocated 5,562,916 USD (1USD=500CFA) for the purchase of 4,147,149 doses of the different presentations of ACTs. The rate of subvention was 36.5% for the combination AS-AQ and 67.15% for the combination AL to allow for whole sale pricing equilibration. Subsidies were fixed by decision number 03621/D/MSP/CAB of 05 February 2007 to vary from 140FCFA (28US Cents) to 600 FCFA (1.2USD). The mechanism is expected to take into account the final costs fixed by the Ministry of Health. This arrangement leaves the following mark-ups for ACTs.

- CENAME to Provinces 10%,
- Provincial Distributors to Health Facilities – 8% and
- Health facilities to Patients – 10%

ACT supplies to public and private sectors 2007

Supplies of ACT to public and private sectors in 2007 are 89.7% and 10.3% respectively. These stocks were depleted within 4 months of deployment. This depletion was slower than stocks of Artemether-Lumefantrine (AL) which just "sublimed" for reasons of perceived side effects of AS-AQ and the flight to neighbouring countries of AL.

Private distribution networks

There are 10 private wholesalers supplied by CENAME. These include 5 mission bodies and 5 sub-wholesalers. The Cameroon Baptist Convention and the Presbyterian Church in Cameroon buy drugs from many international institutions in addition to the government-owned drug reseller CENAME and resell to their satellite institutions, hospital and health centres with a 10-20 % mark-up who themselves add an additional of 30% mark-up to the price charged to patients but do not receive or sell the ACTs at comparable prices to those provided by the South West Regional Special Fund for Health. Its customer prices are 2 times more than that for the government public health facilities.

The catholic health service has a central drug purchase service and buys only registered drugs that are on the EML and sells to its peripheral services through its regional outlet. It does a 35% mark-up for its regional distributors and the church's medicine outlets do a further 15% mark up. If drugs are imported donations, they do a 13% mark-up at wholesale and a further 20% mark-up at retail.

The black market parallel distribution unit has many sources. Drugs are purchased from India, Pakistan and Nigeria. Some pharmacies whose drugs are about to expire sell their drugs through these network. The black market supplies ACT to a wide variety of retailers: some of who are faith-based or non-profit making health facilities, some private profit making pharmacies and patent medicine store retailers and most often to neighbouring countries where these drugs are not subsidized.

Actual prices paid

The following diagram just indicates 500FCFA and 2000FCFA as the dominating prices per pack (Figure 3).Monotherapies were still found in the course of the survey and so are represented in the diagram below (Figure 41). In addition there is not a dominant name in the market but quinine seems to be slightly more present that the others.

Mean price per tablet of ACTs from drug outlets

The survey revealed that the Centre region always had the lowest pricing per drug than the South-West or North-West regions perhaps reflecting the distance to the regions (Figure 4). Prices for example varied between 26F ($0.05) and 333($0.67) per tablet for AS-AQ under different trade names. In 80% of private drug outlets, “convenient packs” (with incomplete doses) were sold as per the purchasing strength of the client. Subsidy benefits seem to work only in Yaoundé with the lowest cost per tablet and particularly for Atemether Lumefantrine in all three regions surveyed (Figure 4).

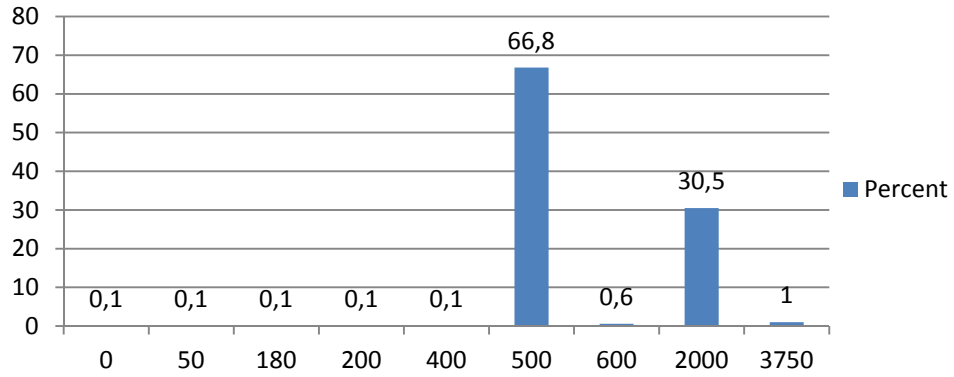


Figure 3. *Pricing per “Pack” in FCFA*

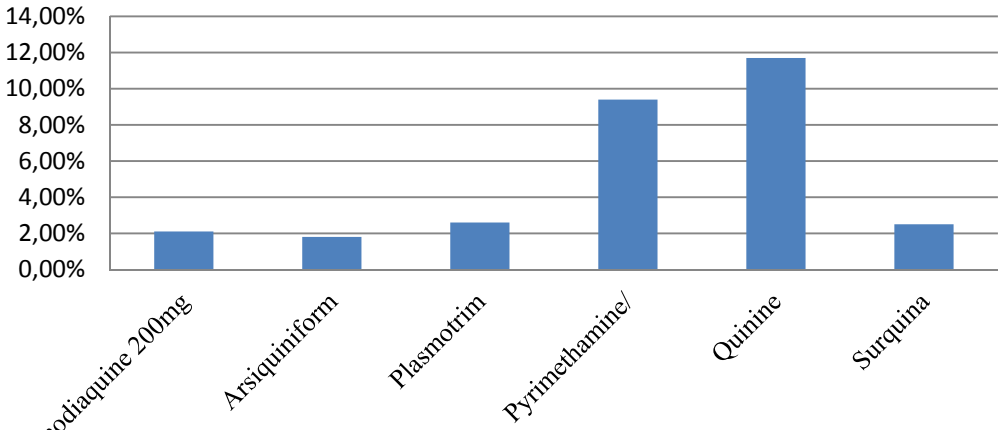
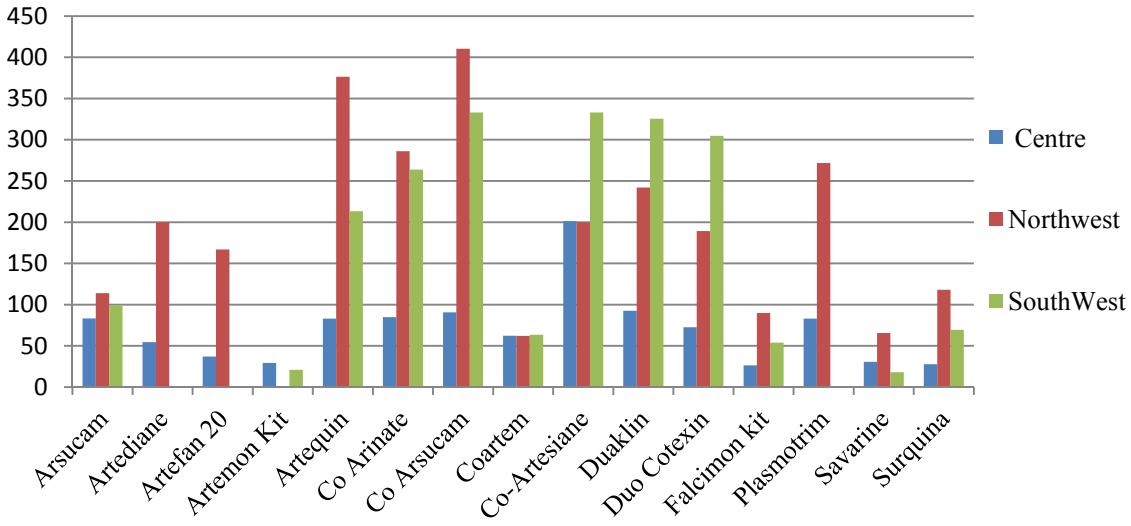


Figure 4. *Frequency of anti-malarials found in the different drug outlets*



The Quality of Anti-Malarials and Purchase Supply Management Challenges in Cameroon

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Introduction

Quality assurance is a wide-ranging concept covering all matters that individually or collectively influence the quality of a product. It is the totality of the arrangements made with the purpose of ensuring that pharmaceutical products are of the quality required for their intended use. *Quality Control* means all measures taken, including the setting of specification sampling, testing and analytical clearance, to ensure that starting material, intermediate, packaging material and final pharmaceutical products (FPPs) conform with established specifications for identity, strength, purity and other characteristics. The Department of Pharmacy and Drugs of the Ministry of Public Health is in charge of enforcing the Quality Assurance policy of the country.

In relation to antimalarials, two ACTs were recommended in 2007 (Artesunate-amodiaquine (ASAQ) and artemether- lumefantrine (AL)) based on WHO 2006 guidelines and on therapeutic efficacy tests carried out in Cameroon. These ACTs were made available and affordable in the public sector whereas, no clear guidelines were given for the private sector. On the other hand, over 90 anti-malarials were authorized for sale in the private sector among which only about 50% conformed to the treatment guidelines. This has greatly increased the risk and the frequency of non-quality assured ACTs and other anti-malarials.

The situation is as severe in the public sector as in the private sector. According to one study, anti-malarials were tested on the field from both public and private sources and revealed that 38% of chloroquine, 74% of quinine and 12% of antifolate (SP) products had no active ingredient, insufficient active ingredient and the wrong ingredient or unknown ingredient(s). The significance of this is the risk of treatment failures, development and spread of resistance and increased malaria related morbidity and mortality.

Report on “Quality of Anti-Malarial Medicines of Selected African countries” (QAMSA) survey

Cameroon was chosen among 6 other countries for a survey titled « Quality of Anti-Malarial Medicines of Selected African Countries”. This was conducted by the National Laboratory for Quality Control (LANACOME) with the support of the NMCP from April to May 2008. The study’s objectivewas the evaluation of the quality of selected anti-malarials in six countries of sub-Saharan Africa (Cameroon, Ethiopia, Ghana, Kenya, Nigeria and the United Republic of Tanzania). Some of the specific objectives were to estimate the proportion of ACT and sulfadoxine/pyrimethamine (SP) products meeting specific quality standards at different points of the regulated and informal distribution systems and to estimate the proportion of counterfeit ACT and SP products at different points of the regulated and informal distribution systems. The causes of findings, strategies and implementation plans to address the problems also had to identified.

A total of 935 samples were collected according to national sampling plans from different distribution levels including the informal market in at least three geographical regions of high malaria prevalence. These were screened using GPHF-Minilab® kits and based on predefined criteria, 306 samples (from 64 manufacturers and 218 sampling sites) were selected for full quality control testing in the WHO prequalified laboratory. Testing was coordinated by the WHO, using the International Pharmacopoeia and data was collected in such a way as to relate the results of quality testing to distribution levels, geographical regions, domestic production or import, registration status and prequalification status.

The results of the study were as follows

- Of 306 samples selected for laboratory testing, 267 were fully tested and 28.5% of them failed to comply with specifications.
- Focusing only on extreme deviations from specifications (as defined in this report) which are likely to be associated with health implications, the failure rate reached 11.6%.
- The failure rate ranged from 10% to about 30% in the six sites sampled.
- Samples obtained from public health facilities had a failure rate almost three times that obtained from private sources.
- Finally, 84% of the failure rate was due to change in the physical characteristics of drugs, while assay and dissolution were each responsible for 8% of the failures.

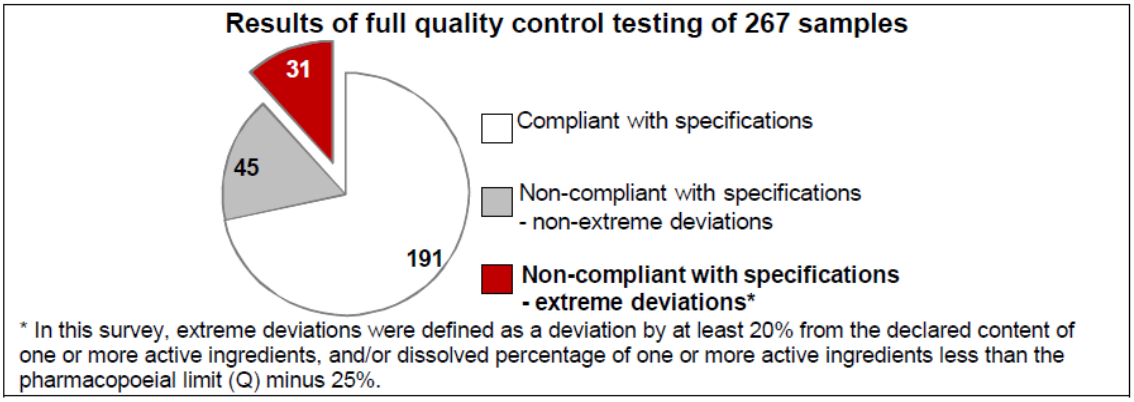

Figure 1. Results of full quality control testing of 267 samples in the QAMSA survey

In Cameroon specifically, failure rates following testing concerned more of SP (>40%) and AL (40%) samples. Less than 10% ASAQ samples were sub-standard.

The study clearly demonstrates the gravity and extent of the problem and shows the need for effective QA policies to be adopted and implemented.
Cameroon has developed several Quality Assurance guidelines and activities in this domain are described in documents such as the National Pharmaceutical Policy, the Procedure Manual of the National Warehouse (CENAME) and Regional Warehouses (CAPRs) and Procurement and Supply Management (PSM) documents of Public Health Programmes.

Quality Assurance activities are taken into consideration throughout the PSM cycle. At each level of the cycle, there should be specific guidelines on quality control principles. Supply sourcing should consider the status of the product (prequalified, GMP or AMM from Stringent authorities), transport requirements (temperature, humidity, packaging, etc), transit conditions, (Douala and Yaounde ports), control at reception and quality control by a certified laboratory.

As far as anti-malarials are concerned, after delivery at the central warehouse, the NMCP monitors the following aspects to ensure quality storage conditions at central and peripheral levels: distribution channels, pharmaceutical waste management, pharmacovigilance and sensitization on rational drug use.

In addition to these activities, other recommendations have been made to improve QA in Cameroon. These include:

- Elaborating a QA Plan to describe measures in place to maintain quality and propose corrective measures to address issues (Inter-agency Quality Assurance policy);
- Defining corrective measures with timeframe, indicators and budget;
- Creating QA Committees for the follow up of measures and regular update of the QA Plan;
- Creating a platform which includes IGSP, CENAME, LANACOME, DPM, PNLP, DGD, DGSN, MINDEF/SED, MINATD, professional councils, private sector, consumers and manufacturers to coordinate QA activities;
- Creating and render functional a multi-sectorial brigade for the control of the sale of anti-malarials and other drugs;
- Elaborating, adopting and distributing a legal framework for implementation of QA and market surveillance;
- Ensuring capacity building of all actors.

The following challenges need to be considered when implementing the above-mentioned recommendations:

- Multiple sectors need coordination by the government;
- Porous borders need reinforced control;
- Governance;
- Insufficient communication;
- Insufficient qualification of persons involved in control.

Quality Assurance is a crucial strategy in the fight against the development and spread of resistance. The NMCP has a major role in implementing these recommendations. However, there are other principal actors such as the DPM and the IGSP that have to be committed to the process and give appropriate technical support to the NMCP. Several sectors are involved and need to be brought on board to implement a national QA plan.

References

1. *Basco,2004*
2. *QAMSA survey 2008*
3. *Interagency QA policy 2012*
4. *QA policy of the Global Fund*

Cameroon's Initiative to Avoid Stock Outs of ACTs

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The Cameroon Health System is divided into 10 regions. These regions are further divided into health districts which are also divided into health areas. There are malaria control units at the regional levels. The heads of units serve as control managers at the regions. The distribution of drugs to the various levels of the health system faces a lot of challenges as a result of bureaucracy and poor reporting mechanisms. There is also inadequateinformation on the epidemiology of the disease which can help to assess the burden of the disease and the likelihood of use of drugs.

Strategies to avoid stock outs

In order to avoid stock outs, it is necessary to identify the needs through quantification based on epidemiological data than on consumption data. The strategies to avoid stock outs include:

- a concerted effort at mobilising additional funding for the acquisition of drugs;
- elaborating guidelines for the proper use and management of ACTs;
- the training of actors involved in drug management (pharmacy attendants, district medical officers, chiefs of health centres) on stock management;
- the supply of stock management tools to health facilities (through CAPR).

These stocks must be monitored based on health facility reporting forms, CAPR data and NMCP data base to access consumption at all levels. Supervision will provide periodic inventories of ACTs. Procurement of ACT funded by Global Fund through the VPP requires on average of 6-8 months to deliver.This makes the procurement through the VPP in Geneva from funds of the Global funds a major challenge that African countries battle with.

Implementing the Strategies

To implement these strategies, the needs for ACTs must be estimated up to 2 years in advance and the quantities are updated annually based on recent morbidity and consumption data. However, the many funding sources for ACTs(State of Cameroon, Global Fund, bilateral partners such as China)has resulted in the subsidy of ACTs and a flexibility in procurement in case of problems with one funding body.

Guidelines for the proper use and management of ACTs are available. They are updated when necessary, printed and distributed at all levels. These documents were elaborated in 2010 and updated in 2013. In 2007, 542 members of District Health Management Teams (DHMT) and 2029 pharmacy attendants were trained on the correct management of stocks of antimalarial drugs (ACTs) nationally.Coordination workshops were also carried out for all managers of Drug Supply System (CENAME, CAPR, Private sector, DRSP,…) in 2013 to improve stock management of anti-malarials.

The supply of stock management tools such as stock cards and monthly forms are distributed regularly to health facilities (through CAPR). For the follow up of ACTs and other anti-malarials, there is monthly collection and analysis of information at CENAME and CAPRs by the NMCP. Such information includes batch number, expiry date, quantity available at the beginning and end of the month and number of months of consumption (according to the average monthly consumption).

Supervision

A supervision checklist includes a section on the ACTs in the health facility (availability and use of management tools, consumption, stock outs, evaluation of the capacity of the health facility to estimate its needs, management of funds, etc.). This supervision is important because thelow financial capacities of some health facilities do not allow them to procure adequate quantities despite the subsidy. In addition there may be preference by health facilities to procure or sell ACTs with higher benefit margins (and which are more expensive than subsidized ACTs).

Development Inadequacies of Low and Medium Income Countries

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Representative for Research & Academia, RBM Partnership Board

Introduction

In the history and dynamics of anti-malarial drug resistance, it has sometimes taken less than a year for resistance to develop against drugs that took 15-20 years to develop (e.g. Atovoquone, pyrimethamine). We do not know how long it will take for the new Artemisinines to become obsolete. Experience demonstrates that the loss of monotherapy which started in South East Asia would ultimately spread to Africa and therefore the monitoring of the situation in Asia serves as a sensor for the African continent. This loss of monotherapies has forced the WHO to suggest that combination therapy be adopted in endemic countries to save the Artemisinines. Currently more than 34 countries in Africa use some form of an ACT therefore exposing some 651 million Africans to the drug. At the current rate of above 95% efficacy, some 34 million people are refractory for one reason or the other to these drugs. That is a huge enough pool when added to those of SE Asia to begin to be suspicious about the eminent upsurge of 'resistance'. Already reports indicate that the time to clearance of parasites after administration of ACTS has risen in SE Asia. It means that the ACTs are taking longer than originally observed to clear parasites from the host.The troubling aspect about these parasites is that it is difficult to say exactly which of the parasites would show this new phenomenon because the genetic diversity of the parasite is enormous but is predictable except that the local pressures could generate unprecedented upsurge of uncertainties. As previously shown, the genetic makeup of the parasite is so different in various regions that common or universal strategies may not work. It is the same phenomenon observed for the diversity of HIV across Africa to the extent that it affects the development and evaluation of interventions such as: tracking the dynamics and spread of genetic subtypes of HIV-1 and CRFs on a global basis, the generation of key data to provide rationale for matching the locally prevalent HIV strains with vaccine candidates and the provision of critical information for improved diagnostic and treatment strategies. Other scientific challenges include the correlates of immune protection that are non-sterilizing, the role and value of animal models that is so badly needed and the multiple vaccination strategies that need to be explored. The situation is similar with challenges for malaria vaccine R&D that include increased antigen diversity, the lack of correlates of protection, the lack of animal models, the lack of comparators (endpoint, assays, trial design) and the lack of blood stage challenge model.

LMICs Health in Peril

Africa has certain challenges to health. These include limited resources that are directed to other emotional needs and often misguided towards political strategies. Limited resources do not favour proper budgeting and rational thinking with the result that we seek for alternative solutions since the health gains are not directly palpable. With the African agenda dictated by war, famine and natural disasters, the mobilisation of funds for health is too slow, too late and too little. Therefore, improvements are needed on its imperfect tools and insufficient knowledge or unused knowledge to

ensure stronger adequate health systems and infrastructures. Cameroon and Africa will have to learn to change habits amid other competing health and social priorities and adopting a more biological-based perception ofdisease and health. This is particularly important as malaria is no longer a disease that occurs in isolation becausethere is increasing prevalence of respiratory tract infections, bacterial infections, congenital diseases andnon-communicable diseases such as cancers, cardiovascular disease, obesity, etc which confound the picture.

Evidence points to the fact that there has been an increase in non-communicable diseases. In Ghana for example the risk of cardio-vascular disease is 32% in urban as against 10% in rural. Of the 177 million with diabetes Type II, 65% are in less developed countries. This is projected to increase to 70% by 2020. About 70% of cardiovascular disease deaths and 50% of deaths attributed to other chronic diseases are due to smoking, unhealthy diet, inactivity and high blood pressure. On the global scale, in 2007, 7.6 million cancer deaths occurred as against 5.6 million for Malaria, HIV and TB combined. Between 2000 and 2030, diabetes is expected to rise from 171 to 366 million with more than 75% to come from LDC. These non-chronic non-communicable diseases only help to worsen the situation for malaria control because they exacerbate the symptoms and make malaria control and intervention efforts a nightmare.

Given the above, it is imperative to know the malaria nemesis by going back to the basics - basics biology, basic epidemiology and basic operations. These and other basic observations placed Africa at the centre of a new kind of research centred approach to health care provision and forced the Ministers of Health last June in Algiers to take a step back to redefine the kinds of research for health agenda that Africa should embark on.

LMICs Health in Peril

The Algiers declaration can be regrouped into 4 main areas:

- The need for Africa to recreate an enabling environment with improvement in infrastructures, encouragement of brain gain, establishment of network of researchers and the installation of scientific and ethics oversight bodies;
- The support of reference Centres of Excellence;
- Rebuilding the public trust in research; and
- The encouragement of N-S and S-S synergies.

The second area of focus is that of knowledge management where she encourages her scientists and institutions to:

- Set norms and standards in GCLP and ethics;
- Harness new technologies for impact on development;
- Provide evidence for policies;
- Improve access to scientific information;
- Strengthen Health Information System;
- Approach health with the understanding of its multi-determinants.

The third focus is for its scientists to:

- Be involved in fundamental or basic research;
- Addressissues such as genome comparisons through field characterisation, the characterisation of functional proteins and their tissue localisation;
- Be involved in systems biology;

- Develop platforms and assays for validating candidate vaccines;
- Use biomimetics and kinetic assays for phytochemical discoveries, the definition of immune correlates and modulators;
- Usethe latest information technology to foster bioinformatics and genomics.

The fourth area to get committed to is that of:

- Operational and translational research requiring that Africa sets out to
    - Be GCLP competent,
    - Conduct cost-effectiveness analysis of interventions,
    - Master the basic epidemiology of the changing patterns of morbidity and mortality through disease registries,
    - Improve on health systems,
    - Investigate efficacy, safety and the tracking of resistance due to complications from MDR TB, MDR Pf, ART-Refractory HIV,
    - Master the vector geography for vector-borne diseases,
- Appreciating the extent of confounder non-communicable diseases variables which are on the rise.

Cameroon like Africa needs to let experience and observations generate the research questions and drive the science. The research products need to be use-inspired and also of fundamental nature.Sometimes these require evidence that is difficult to capture with existing indicators. For any advocacy drive to be effective, we need to:

- Collect the right data;
- Capture complexity in current practices;
- Acquire beneficial technologies ;
- Maintain high infrastructural investment;
- Develop better funding programmes;
- Encourage multidisciplinary teams;
- Improve on good governance.

Perspectives on the WHO Global Report on ID

The WHO in 2010, through a consultative group on ID, suggested 10 compelling reasons why research on infections of poverty must continue. These include:

1. Suggesting ways to break the vicious cycle between poverty and infectious diseases;
2. Forging an escape route for the poor and vulnerable;
3. Providing solutions for tackling multiple problems at the same time;
4. Finding ways to mitigate life-long chronic illnesses and stigma;
5. Stepping up surveillance at all levels to pre-empt emergence of resistance;
6. Reaching out to the hardest to reach population through health systems improvements, etc;
7. Preventing loss in translation;
8. Identify small changes that can make big differences;
9. Focus on light at the end of the tunnel.
10. Acting on what we know now.

Five high level actions were recommended:

i. Create and use a new "index of infectious diseases of poverty" to serve as a surrogate marker of national socioeconomic development;
ii. Implement a "One Health, One World" strategy in relations to research for infectious diseases of poverty;
iii. Actively promote research ownership with enabling policies by diseases endemic countries;
iv. Create an innovation platform to foster a culture of innovation to benefit public health;
v. Create an online global platform of research resources to inform on strategies, policies and funding commitments.

Recommendations from the WHO consultative group on R&D required that African countries be involved in:

- Innovative approaches to R&D with open knowledge innovation as well as equitable licensing and patent pools;
- Funding for R&D whereall countries are to devote 0.01% of GDP on gov't-funded R&D product development in LMICs and a proportion of 20-50% of the funds should be devotedto health R&D through a pooled mechanism;
- Strengthening R&D capacity and technology transfer where they address the capacity needs of academic and public research organizations in developing countries and also utilize direct grants to companies in developing countries.

Illustrative efforts: is big Pharma to the rescue?

The Big Pharmas in Europe and America combined forces with the WHO and BMGF, to conduct the eradication of 10 neglected tropical diseases by 2020 affecting 1.4 billion people in the world's poorest countries. Together with thirteen pharmaceutical companies they contributed $785 million to tackle blinding trachoma, leprosy, chagas disease, sleeping sickness, leishmaniasis, guinea worm, lymphatic filariasis, river blindness, schistosomiasis and yaws. Besides these, other corporate social responsibility initiatives have developed different models to address these problems:

MMV Model

- Exclusivity**:** to develop a drug for malaria and bring it to market.
- Royalty-free**:** to help keep costs to a minimum and ensure that the drug will be sold at the lowest price possible in these countries.
- Transferable: requires IP rights that can be transferred to other partners – especially manufacturing partners - if necessary

SANOFI Model

- With DNDi -**No** Profit **No** Loss**:** Drugs (ASAQ) are manufactured and sold at industrial cost of manufacture.
- African Continental Presence**:** To boost Africa's capacity to perform in GMPs
- Train Care Givers**:** to ensure proper use of limited drug options
- Assistance of NMCP with Pharmaco vigilance

Novartis Model

- Novartis Institute for Tropical Diseases
- GSK Training Program in Vaccinology
- Sanofi's training courses in Madagascar and Tanzania
- Sanofi's Annual Meeting of National Malaria Control Programme Managers – APALP

LMICs need to re-position with a common and unified approach to R&D through common resource allocation platform, the adoption of a performance mentality by mastery of the steps in drug discovery and investment in research infrastructure to be attractive for the outsourcing of innovative technologies from the north. When African countries reposition, it is evident that they can pool easily from Big Pharma who already are orienting efforts towards investments to target the grassroots by assisting LMICs build the research sector, tapping the brain power of the new generation of LMICs genome engineers, investing more in NTDs and filling up the gap on PRDs and rebuilding new molecules from old ones through synthesis chemistry.

Recommendations

1. The overuse of ACTs within the private sector that can occur when malaria treatment is provided to patients who do not have malaria can lead to the selection of resistance parasites. The emergence of resistance to Artemisinines has already been reported in South East Asia. The loss of ACTs as an efficacious malaria treatment would severely jeopardize efforts to control and ultimately eliminate malaria in Sub-Saharan Africa. Spread of this resistant parasite to Sub-Saharan Africa would represent a public health catastrophe. Pursuing the policy of presumptive therapies may be temporarily cost-effective but may ultimately lead to long-term increase in parasite resistance. *Consequently, it is recommended thus:*

- *There is need for regular monitoring of resistance to currently used first line antimalarial drug choice in designated sentinel sites to detect possible development of drug resistance.*
- *The rational use of ACTs only in patients with confirmed malaria is critical so that the useful lifespan of ACTs and other currently available antimalarial drugs can be maximized. The malaria control programme should prepare and widely disseminate a treatment manual for malaria to all health centres to ensure compliance with treatment guidelines thus reducing the arbitrary use/abuse of anti-malarials.*
- *Treatment must be based on the parasitic demonstration of Plasmodium spp through the use of Rapid Diagnostic tests (RDTs) that allow ultrasensitive same-day, on-site diagnostics in hundreds of patients or microscopy.*
- *Peripheral laboratories should be equipped with rapid diagnostic test kits to facilitate diagnosis and target therapy of febrile cases.*
- *The personnel should also be trained.*
- *Training schools in the field of health need to be brought up to date on these guidelines.*

2. In order to help public health officials adopt evidence-based health policies for malaria control in Cameroon, the workshop participants called on researchers to:

- *Elucidate the pharmacokinetics and pharmacodynamics and optimal dosing of drugs used to treat and prevent malaria, especially in understudied vulnerable groups such as pregnant women, young children and infants;*
- *Develop, standardize and preserve Cameroon's traditional pharmacopeia in order to generate new molecules for treating malaria;*

- *Address malaria within the context of One Health concept where the environment, animal and human health are equally weighted and dealt with in an integrated manner as pieces of the same puzzle.*

3. Recently, there has been the identification of the monkey malaria, *P. knowlesi*, a widespread and potentially life-threatening human pathogen. This suggests that *vigilance for transfer of other nonhuman primate malarias to humans and the determination of which drugs are effective against these emerging diseases are necessary.*

ANNEXES

Discours d'ouverture de Madame le Ministre de la Recherche Scientifique et de l'Innovation

Monsieur le Ministre de la Santé Publique
Chers collaborateurs,
Mesdames et Messieurs

C'est un honneur pour moi de présider la cérémonie d'ouverture de cette conférence sur la « Résistance des Antipaludéens au Cameroun ».

Je salue ici la présence du Ministre de la Santé Publique dont la présence indique l'importance accordée à la recherche des connaissances scientifiques et technologiques pour l'amélioration de l'état de santé des Camerounais menacés par le paludisme et engagés dans la voie de l'émergence à l'horizon 2035.

Je remercie l'Académie Nationale des Sciences des Etats Unis d'Amérique (US NAS) qui a financé la tenue de cet atelier ainsi que l'Académie des Sciences du Cameroun (CAS) qui a intelligemment choisi le thème sur « la résistance des souches aux médicaments contre le paludisme » en ce début d'année 2013.

Au moment où notre économie est de plus en plus sollicitée dans différent secteurs par les investisseurs nationaux et étrangers, il convient de dresser un point essentiel sur les médicaments contre le paludisme pour garantir aux populations la santé sans laquelle aucun capital humain ne peut exister et sans laquelle le développement ne peut fleurir au plan local, sous régionale et continentale.

Les récentes études en Afrique révèlent que les défis qui l'interpellent l'obligent à demeurer au rang de continent aux nations les plus pauvres de la planète de par son PIB. Cette position est davantage renforcer au sort qu'inflige le paludisme aux populations de ces territoires qui se livrent à la prise élevée des antipaludéens et à la médecine traditionnelle ; à l'influence de la culture et à la conviction que la maladie est d'origine mystique et ancestrale.

A la veille de l'atteinte des Objectifs du Millénaire pour le Développement (OMD), il convient de rappeler que des 8 objectifs visés, 6 ne seront pas atteints si le paludisme n'est pas maitrisé. Et le continent restera donc pauvre et affamé, avec une morbidité et mortalité infantile élevée, l'éducation des enfants non accomplis, la santé maternelle non améliorée ; la lutte contre le SIDA, paludisme et autres maladies sans progrès ; la coopération internationale et le partenariat non développé à l'ère de la globalisation.

Au Cameroun et en Afrique, des études sur la « Résistance aux Marqueurs Moléculaires » ont permis des avancées considérables. L'on sait donc déjà qu'il existe une différence entre les *Plasmodium falciparum* d'Afrique de l'Ouest et celui de l'Afrique de l'Est et par conséquent ; la résistance aux antipaludiques s'est développée indépendamment dans les multiples sites et coins d'Afrique ces derniers 10 à 20 ans. Ce qui complique de plus bel l'espoir d'une maitrise de la résistance des souches aux antipaludéens.

Avec le passage de la monothérapie à la combinaison thérapeutique depuis 2005 au Cameroun ; et la tendance de l'Organisation Mondiale de la Santé (OMS) d'introduire les tests de diagnostiques rapides (TDR) depuis 2010 pour juguler les causes de la résistance aux antipaludéens, l'on note le désir des scientifiques et de la communauté contre le paludisme de mettre fin à ces pratiques qui détruisent l'innovation pharmaceutique et s'érigent contre le bien-être social recherché par le gouvernement.

Mesdames et Messieurs,

Ces quelques points sus cités et d'autres que vous véhiculez sont des indicateurs qui interpellent une actions plus rigoureuse et attentionnée des différents acteurs impliqués dans cette lutte au 21^{e} siècle. Pendant ces deux jours, une connaissance scientifique basée sur l'échange d'expertise de cette problématique permettra de marquer un nouveau pas vers les progrès escomptés sur la résistance des souches aux antipaludéens.

Chers participants,

Je fonde bon espoir qu'au terme de cette rencontre, les institutions de la santé et les décideurs vont s'approprier de la nécessité effective posée par la résistance aux antipaludéens dans notre pays.

En vous souhaitant de bons échanges, j'adresse également le vœu que les résultats de vos débats conduisent à la formulation des recommandations dont la mise en œuvre permettra de réduire les causes de la résistance aux antipaludéens au Cameroun dans un bref avenir.
Sur cette note, je déclare ouvert l'atelier sur la « Résistance des Antipaludéens au Cameroun ».

Vive la coopération internationale
Vive le Cameroun
Je vous remercie

Programme

Time	Description	Speaker
DAY 1		
08H30 - 09H00	Registration, Welcome and introductory Remarks	CAS President – Pr. Domngang
Session I – Moderator: Prof. VPK Titanji and Alexi Tougordi		
09H00-09H15	The Changing Nature of Malaria Management	Pr. W. Muna Chair Health Forum, CAS, FMBS, UYI
09H15-09H40	Malaria and Development – The MDGs	Dr Esther Tallah, MC-CCAM
09H40-10H00	Malaria Epidemiology and Transmission Potential post-LIN	Dr Jude Bigoga, BTC- UYI
Session II – Moderator: Prof. V.P.K. Titanji		
10H30-10H50	Evolution of Malaria Treatment Guidelines in Cameroon	Dr. Dorothy Forsah-Achu, NMCP - MINSANTE
10H50-11H10	Prescriber Behavior - REACT Study Results	Pr Wilfred Mbacham
11H35- 11H55	DISCUSSIONS	
11H45	Arrival of Minister of MINRESI and Remarks	Pr. Samuel Domngang President CAS
12H00	Statement from the Minister	H.E. Madeleine Tchuinte MINRESI
12H10	Family Photo and Lunch	Mr. Ego, CAS
Session III – Moderator – Dr Victor Ndiforchu		
13H20-13H40	Intermittent Preventive Therapy in Pregnancy	Pr Rose Leke, BTC FMBS, UYI
13H40-14H00	Methods in Malaria Research - Efficacy & Resistance: *In vitro* Evidence of resistance	Dr. Palmer M. Netongo BTC, FS, UYI
14H00-14H20	Modeling to contain Anti-malaria Drug Resistance	Dr. Akindeh Nji, BTC, FS, UYI
14H20-14H40	DISCUSSIONS	
14H40-15H00	COFFEE BREAK	
Session IV – Moderator: Dr. Victor Ndiforchu		
15H00-15H20	Quinine overuse and Efficacy	Prof Eric Achidi, UB
15H20-15H50	Drug Resistance Profile in Cameroon	Pr. Wilfred Mbacham BTC, FS, UYI
15H50-16H10	A Decade of ACT Efficacy in Cameroon	
16H10-16H40	DISCUSSIONS	Moderator
DAY 2		
Session V – Moderator : Prof. Wali Muna		
09H00-09H10	Recap of previous days discussions	Rapporteur
09H10-09H30	Subsidies to ACTs and Cameroon Experience	Dr Palmer Masumbe Netongo
09H30-09H50	The Quality of Anti-malarials	Dr Dorothy Forsah-Achu
10H10-10H30	Cameroon's Initiative to avoid Stock outs of ACTs	Dr Celestin Kouambeng
10H30-11H00	DISCUSSIONS	
11H00-11H20	Closing Editorial - Of Drugs & the Malaria Eradication Agenda	Wilfred Mbacham, scD Workshop Chair
11H30	Concluding Remarks and close of workshop	Prof. W. Muna, Chair Forum, CAS, FMBS, UYI
12H30	Departing lunch	