Gharbi, Myriam; Flegg, Jennifer A; Pradines, Bruno; Berenger, Ako; Ndiaye, Magatte; Djimdé, Abdoulaye A; Roper, Cally; Hubert, Véronique; Kendjo, Eric; Venkatesan, Meera; +7 more... Brasseur, Philippe; Gaye, Oumar; Offianan, André T; Penali, Louis; Le Bras, Jacques; Guérin, Philippe J; Members of the French National Reference Center for Imported Mal; (2013) Surveillance of travellers: an additional tool for tracking antimalarial drug resistance in endemic countries. PloS one, 8 (10). e77775-. ISSN 1932-6203 DOI: https://doi.org/10.1371/journal.pone.0077775

Downloaded from: http://researchonline.lshtm.ac.uk/id/eprint/286948/

DOI: https://doi.org/10.1371/journal.pone.0077775

**Usage Guidelines:**

Please refer to usage guidelines at https://researchonline.lshtm.ac.uk/policies.html or alternatively contact researchonline@lshtm.ac.uk.

Available under license: http://creativecommons.org/licenses/by/2.5/
Surveillance of Travellers: An Additional Tool for Tracking Antimalarial Drug Resistance in Endemic Countries

Myriam Gharbi1,2,3,4**, Jennifer A. Flegg3,5, Bruno Pradines6,7,8, Ako Berenger9, Magatte Ndiaye10, Abdoulaye A. Djimdé11, Cally Roper12, Véronique Hubert13, Eric Kendjo14, Meera Venkatesan3,15, Philippe Brasseur16, Oumar Gaye10, André T. Offianan9, Louis Penali3, Jacques Le Bras12,3,13, Philippe J. Guérin3,4,5,17, Members of the French National Reference Center for Imported Malaria Study

1 Unité Mixte de Recherche 216, Institut de Recherche et de Développement, Paris, France, 2 PRES Sorbonne Paris Cité, Faculté de Pharmacie, Paris, France, 3 WorldWide Antimalarial Resistance Network, Oxford, United Kingdom, 4 École des Hautes Études en Santé Publique, Sorbonne Paris Cité, Rennes, France, 5 Centre for Tropical Medicine & Nuffield Department of Clinical Medicine, University of Oxford, Oxford, United Kingdom, 6 Département d’Infectiologie de Terrain, Institut de Recherche Biomédicale des Armées, Marseille, France, 7 Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes, Aix Marseille Université, Marseille, France, 8 Centre National de Référence du Paludisme, Marseille, France, 9 Malariaology Department, Institut Pasteur de Côte d’Ivoire, Abidjan, Côte d’Ivoire, 10 Service de parasitologie, Faculté de Médecine et Pharmacie Université Cheikh Anta Diop, Dakar, Sénégal, 11 Malaria Research and Training Center & Department of Epidemiology of Parasitic Diseases, Faculty of Pharmacy University of Sciences Techniques and Technologies of Bamako, Bamako, Mali, 12 Pathogen Molecular Biology Department of Infectious Tropical Diseases, London School of Hygiene and Tropical Medicine, London, United Kingdom, 13 Centre National de Référence du Paludisme & Service de Parasitologie Mycologie, CHU Bichat-Claude Bernard APHP, Paris, France, 14 Centre National de Référence du Paludisme et Service de Parasitologie Mycologie, CHU Pitié-Salpêtrière APHP, Paris, France, 15 University of Maryland School of Medicine, Baltimore, Maryland, United States of America, 16 UMR 198, Institut de Recherche pour le Développement, Dakar, Sénégal, 17 UMR S 707: Epidemiology Information Systems Modeling, INSERM and Université Pierre et Marie-Curie-Paris6, Paris, France

Abstract

Introduction: There are growing concerns about the emergence of resistance to artemisinin-based combination therapies (ACTs). Since the widespread adoption of ACTs, there has been a decrease in the systematic surveillance of antimalarial drug resistance in many malaria-endemic countries. The aim of this work was to test whether data on travellers returning from Africa with malaria could serve as an additional surveillance system of local information sources for the emergence of drug resistance in endemic-countries.

Methodology: Data were collected from travellers with symptomatic Plasmodium falciparum malaria returning from Senegal (n = 1,993), Mali (n = 2,372), Cote d’Ivoire (n = 4,778) or Cameroon (n = 3,272) and recorded in the French Malaria Reference Centre during the period 1996–2011. Temporal trends of the proportion of parasite isolates that carried the mutant genotype, pfcr1 76T, a marker of resistance to chloroquine (CQ) and pfldhfr 108N, a marker of resistance to pyrimethamine, were compared for travellers and within-country surveys that were identified through a literature review in PubMed. The in vitro response to CQ was also compared between these two groups for parasites from Senegal.

Results: The trends in the proportion of parasites that carried pfcr1 76T, and pfldhfr 108N, were compared for parasites from travellers and patients within-country using the slopes of the curves over time; no significant differences in the trends were found for any of the 4 countries. These results were supported by in vitro analysis of parasites from the field in Senegal and travellers returning to France, where the trends were also not significantly different.

Conclusion: The results have not shown different trends in resistance between parasites derived from travellers or from parasites within-country. This work highlights the value of an international database of drug responses in travellers as an additional tool to assess the emergence of drug resistance in endemic areas where information is limited.

Introduction

A decline in artemisinin efficacy has recently been confirmed in several regions in Southeast Asia [1,2,3]. Concerns are growing about the potential for this artemisinin resistance to spread to sub-Saharan Africa, as it has previously been described for other antimalarial drugs. Indeed, resistance to chloroquine (CQ) and
sulfadoxine-pyrimethamine (SP) emerged relatively quickly after their introduction and subsequently spread from Asia to Africa [4,5]. Early detection of decreasing drug efficacy and the consequent updating of drug policies are crucial elements in the strategy to prevent the emergence or delay the spread of drug resistance [6,7]. In recent years, considerable effort has been made to improve epidemiological antimalarial resistance surveillance in countries with limited resources. Therapeutic efficacy studies remain the gold standard for guiding drug policy, as they take into account the complex interactions between the host, parasite and drug [8]. However, many settings in endemic countries lack the financial resources necessary to maintain a sustainable, accurate and reliable antimalarial resistance surveillance system, resulting in gaps in the spatial and temporal available information.

In recent years, globalization and a substantial increase in international travel and population mobility, have provided the potential for the rapid spread of infectious diseases and antimicrobial resistance [9]. More than 900 million international journeys are undertaken annually and this figure has been consistently rising over the years (United Nations World Tourism Organization: UNWTO).

Malaria is endemic in over 100 countries and represents an important infectious disease threat for these nations. Of the 125 million people travelling to malaria endemic countries each year, approximately 10,000 malaria infections were reported worldwide in returning travellers in 2010. Under-reporting is thought to be substantial and, hence, this number may, in reality, exceed 30,000 [10]. In Europe, a 10-fold increase in imported infections was reported from 1970 to 2000 (from 1,500 to about 15,000 cases) before decreasing to about 6,000 cases in 2010 [http://data.euro.who.int/cisid]; most of these cases were reported in France or the United Kingdom [11]. Travellers who return from endemic countries infected with malaria often present with low immunity against the parasites and there is no risk of re-infection, so they are a particularly valuable source of information.

In fact, historically, the emergence of CQ resistance in Africa was mainly detected through surveillance of travellers (Figure 1, Table 1). The current study was undertaken to test the idea that surveillance of parasites from travellers can be used to accurately assess the evolution of antimalarial drug resistance and provide complementary information to existing monitoring. As a proof of concept, the aim was to compare trends in molecular and in vitro markers of drug resistance observed in the imported malaria population with the trends described in field studies.

Materials and Methods

Data Collection

The studies were conducted by the National Reference Centre for Malaria (CNR), Paris, France and investigators from the four endemic countries, in collaboration with the WorldWide Antimalarial Resistance Network (WWARN).

Data from travellers. Data were collected from travellers with symptomatic Plasmodium falciparum malaria returning from malaria-endemic countries during the period 1996–2011. These cases were reported to the French CNR by one of the 80 hospitals participating in the sentinel network for malaria which covers about half of the cases diagnosed in France. All the travellers included in this study must have visited a malaria-endemic African country in the two months prior to diagnosis and presented with a P. falciparum infection biologically confirmed by thin and thick blood smear. Basic demographic, epidemiologic, clinical, and parasitological information as well as response to treatment, previous malaria infection and travel history information were systematically reported. Blood samples were only collected from hospitals which document anti-malarial drug resistance on a systematic basis in all Plasmodium positive diagnosis, before treatment, for molecular and in vitro analyses.

Molecular markers associated with resistance to CQ and pyrimethamine and CQ susceptibility in vitro were the tools used to compare antimalarial drug resistance trends between travellers and field studies.

No informed consent was required for this study as the procedures described here were part of the French national surveillance system of malaria. In 2006, the Commission Nationale de l’Informatique et des Libertés (CNIL), France, approved the electronic-based utilisation of patient-level data collected through the CNR’s questionnaire. We did not receive ethical approval or waiver to perform this secondary research on samples collected as a part of government surveillance for communicable diseases (Loi n° 2004-806 art.16, 21, 9 August 2004, Journal Officiel 11 August 2004). However, ethical aspects have been respected according to the French regulation (article L.1211-2, L.1111-7, L.1413-4 and L.1413-5 Code de la Santé Publique). Also an information note, which explained that the collected blood samples could be used for further research analyses, was provided to the patients who had the possibility to refuse; and the samples were anonymised for this study.

Data within-country. A literature review was performed in PubMed for publications on malaria from African endemic countries during the period 1996–2011. The search terms [country name]+(pfcrt OR chloroquine resistance) and [country name]+(DHFR OR sulfadoxine-pyrimethamine resistance OR sulfadoxine pyrimethamine resistance) were used.

After performing a sample size calculation (see below sample size calculation section), four African countries, Senegal, Mali, Cote d’Ivoire and Cameroon were included in this study. They had sufficiently large numbers of both travellers and field molecular data, from 1996–2011, for a meaningful comparison.

The collaboration of investigators within the four targeted countries, facilitated by WWARN, enabled the collation and standardisation of published field data and the identification and standardisation of unpublished field data. The field studies used for the analyses are summarized in Table 2.

Laboratory Analysis of Parasites

Molecular analysis. Two molecular markers, pfcrt 76 for CQ resistance and pfdhfr 108 for pyrimethamine resistance, were used in this study to compare the trends between travellers and field data. Although the presence of these two markers does not perfectly correlate with treatment failure, each is a good proxy of the intrinsic resistance of the parasite [12,13]. They are used here as a proof of concept since they have been widely and consistently collected in both field studies and travellers surveillance over the period of interest. Due to the availability of travellers’ data, the time period of 2000–2011 was studied for pfcrt 76T, while the time period of 1996–2011 was used for pfdhfr 108N.

For molecular analyses of parasites from travellers, DNA was extracted from blood samples of P. falciparum, using the QIAamp DNA Mini Kit, Qiagen® before 2008 and the MagNA Pure LC DNA Isolation Kit I, Roche after 2008. PCR and subsequent allele-specific restriction analyses were performed to identify polymorphic codons of interest at the pfcrt 76 locus (Lys to Thr) and the pfdhfr 108 locus (Ser to Asn) [14].

For field studies, the genotyping methods differed slightly between studies. The detailed method for each study was described in the corresponding publication (see Table 2 for the references to the studies).
Only “pure” \textit{pfcrt} 76T and \textit{pf dhfr} 108N infections among the total number of samples tested were included to improve comparability of the allele prevalence calculated between studies. Indeed, genotyping methods for detecting mixed infections vary in sensitivity across studies. There is not clear evidence that patients living in endemic countries are more likely to carry mixed alleles (mutant and sensitive) than travellers returning from endemic countries as the number of mosquitoes’ bites is not the only factor to consider and the presence of mixed infections is possible after only one bite [15].

\textbf{In vitro assay.} For the \textit{in vitro} susceptibility tests, only data from Senegal were analysed in this study because there were sufficient available data for parasites from both the travellers and within-country isolates over the complete period of interest.

For susceptibility tests of parasites from travellers, the following methods were used. The batches of plates were validated on the CQ-susceptible 3D7 reference strain and the CQ-resistant W2 reference strain using the standard 42-hour 3H-hypoxanthine uptake inhibition method in controlled atmospheric conditions in the incubator (5% CO$_2$, 10% O$_2$ and 85% N$_2$) [16,17]. The isotopic microtests were performed, aliquoting 200 µl/well of the suspension of fresh parasitized erythrocytes into 96-well plates pre-dosed with CQ. Radioactivity incorporated by the parasites was measured using a scintillation counter. The CQ susceptibility was calculated as the 50% inhibitory concentration (IC$_{50}$) of CQ of the isolates tested [10,19]. The drug concentration that inhibited 50% of parasite growth (IC$_{50}$) was estimated by using nonlinear regression to fit an inhibitory sigmoid E$_{max}$ model [20]. The \textit{In Vitro Analysis and Reporting Tool} (IVART) enabled the transformation, standardization and analysis of the data [21].

For within-country surveys, the \textit{in vitro} methods differed between studies and were described in the publications, which are referenced in Table 2. However, the measurement of the drug susceptibility of fresh \textit{P. falciparum} parasites was mainly performed by isotopic assays using the \textit{in vitro} 3H-hypoxanthine uptake inhibition method. The \textit{in vitro} CQ susceptibility was determined by a \textit{P. falciparum}:

| Country | Date case | Date published | Country of detection | Reference |
|---------|-----------|----------------|----------------------|-----------|
| Kenya   | 1978      | 1979           | Denmark              | [29]      |
| East Africa (Kenya-Tanzania) | 1975      | 1979           | United States        | [30]      |
| Democratic Republic of the Congo (Zaire) | 1982      | 1983           | United States        | [31]      |
| Burundi | 1983      | 1984           | France               | [32]      |
| Republic of the Congo | 1984      | 1985           | France               | [33]      |
| Cameroon | 1984      | 1985           | France               | [34]      |
| Angola  | 1984      | 1984           | Denmark              | [35]      |
| Gabon   | 1985      | 1986           | United States        | [36]      |
| Benin   | 1986      | 1986           | France               | [37]      |
| Senegal | 1986      | 1987           | Sweden               | [38]      |
| Cote d’Ivoire | 1987   | 1988           | France               | [39]      |
| Mali    | 1987      | 1988           | France               | [40]      |

*The within-country studies which detected the emergence of CQ resistance in other parts of Africa during this time period are not shown here. doi:10.1371/journal.pone.0077775.t001
Table 2. Summary of the molecular and in vitro field studies in the four endemic countries included in the analysis (both published and unpublished).

| Molecular marker analysis | Country | Site | Age population | Year of study | Reference |
|---------------------------|---------|------|----------------|---------------|-----------|
| *Pfcrt* 76                | Senegal | Pikine | ≥5 ya | 2000 | [41] |
|                           |         | Pikine | ≥18 ya | 2001 | [42] |
|                           |         | Thiadiaye | Pregnant women | 2002 | [43] |
|                           |         | Dakar | 3–65ya | 2002 | [44] |
|                           |         | Pikine | ≥3 ya | 2004 | [45] |
|                           |         | Dakar | All | 2004 | [46] |
|                           |         | Thies | All | 2007 | [47] |
|                           |         | Dakar | All | 2009–10 | [48] |
|                           |         | Central Senegal (3 districts : Mbour, Fatick, Bamby) and Southern Senegal (3 districts : Tambacounda, Velingara, Saraye) | <10 ya | 2009–11 | [49] |
|                           |         | Dakar | all | 2010–2011 | Pradines, unpublished data |
| *Pfdhfr* 108              | Mali    | Kolle | <5 ya | 2002–03 | [50] |
|                           |         | Bougoula-Hameau | >6 mths | 2002–04 | [51] |
|                           |         | Bancourma, Monteourou, Bandiagara, Faladie, Koulikoro Ba, Sirakoro-meg, Niena, Kolebougou, Markakoungo, Dimbal, Kafana, Siekorole, Toguel, M'pessoba Banamba, N'debugou | all | 2002–04 | [52] |
|                           |         | Kangaba et kela | >6 mths | 2001–03 | [53] |
|                           |         | Bandiagara, Faladie Kolle Pongenon | all | 2010 | Dijmide, unpublished data |
|                           | Cote d'Ivoire | Anonkoua-koute (Abidjan), Ayamé, Dabakala | all | 2003–08 | Ako, unpublished data |
|                           |         | Bonoua and Samo | <5 ya | 2005 | [54] |
|                           |         | Abidjan (2 districts: Yopougon and Adjame) | Children | 2006 | [55] |
|                           |         | Adzope | Children | 2007 | [56] |
|                           | Cameroon | Maroua, Ndop, Bafoussam, Hévécam | <5 ya | 2000–01 | [57] |
|                           |         | Yaoundé | >12 ya | 2000–01 | [58] |
|                           |         | Garoua, Yaounde, Mutengene | <5 ya | 2004–06 | [59] |
|                           |         | Yaoundé, Mfou (suburb of Yaoundé) | All ages | 2005–08 | [60] |
|                           | Senegal | Dielmo, Sine Saloum | All | 1996–99 | [61] |
|                           |         | Pikine, Tambacounda, Thies | ≥5 ya | 2000–03 | [62] |
|                           |         | Dakar | 3–65 ya | 2002 | [44] |
|                           |         | Dakar | <5 ya | 2006–08 | [63] |
|                           |         | Dakar | all | 2009–10 | [47] |
|                           | Mali    | Tienequebougou | 2–12 ya | 1996 | [64] |
|                           |         | Kidal | All | 1999 | [65] |
|                           |         | Bandiagara | 5–15 ya | 2000 | [66] |
|                           |         | Kolle | <5 ya | 2002–03 | [50] |
|                           |         | Bongoula-Hameau | >6 mths | 2002–04 | [50] |
|                           |         | Kolokani | <5 ya | 2006–07 | [67] |
|                           | Cote d'Ivoire | Yopougon | <5 ya | 2000–01 | [68] |
|                           |         | Anonkoua-koute (Abidjan), Ayamé, Dabakala | all | 2003–08 | Ako, unpublished data |
|                           |         | Bonoua and Samo | <5 y | 2005 | [54] |
|                           |         | Abidjan (2 districts: Yopougon and Adjame) | Children | 2006 | [55] |
|                           |         | Adzope | Children | 2007 | [56] |
|                           | Cameroon | Bertoua, Douala, Eseka, Yaounde | <5 ya | 1999 | [69] |
**Table 2.** Cont.

| Country                  | Site          | Age population | Year of study | Reference |
|--------------------------|---------------|----------------|---------------|-----------|
| Bafoussam, Bertoua, Djoum, Garoua, Hevecam, Manjo, Maroua, Mengang, Ndop, Ngoundere, Sangmelima, Yaounde | <10 ya | 1999–03 | [70] |
| Di chang, Fontem, Limbe, Nkambe | <10 ya | 2002–03 | [71] |
| Garoua, Mutengene, Yaounde | ≤5 ya | 2004–06 | [59] |
| Yaounde                   | ≥12 ya | 2001–05 | [72] |

**In vitro susceptibility test**

| Drug       | Country | Site          | Year of study | Reference |
|------------|---------|---------------|---------------|-----------|
| Chloroquine| Senegal | Mlimnp        | 1996–98       | [73] |
|            |         | Pikine, Dielmo, NDiop | 1996       | [74] |
|            |         | Dielmo, NDiop   | 1997         | [75,76] |
|            |         | Pikine         | 2000         | [41] |
|            |         | Pikine         | 2001         | [42] |
|            |         | Dakar          | 2002         | [44] |
|            |         | Dakar          | 2009–2010    | [77] |

**Statistical Analysis**

**Sample size calculation.** In order to select eligible countries with enough data per year for significant molecular analysis, a sample size calculation was first performed. The basic comparison of the trends used a simple logistic regression model. The prevalence of isolates from traveller samples that carried a mutant allele (\(P_t\)) or from studies on field samples (\(P_f\)) was the metric used. In the models: \(S\).

\[
\text{logit}(P_t) = a_t + S_t X
\]

\[
\text{logit}(P_f) = a_f + S_f X
\]

where \(X\) is the time covariate and \(a\) the intercept, the null hypothesis of equal temporal slopes was tested [22]. That is,

\(H_0\) : slope in travellers’ data (\(S_t\)) = slope in field data (\(S_f\))

A two sided t-test with a test significance level of \(\alpha = 0.05\), a power of \(1 - \beta = 0.80\) and an effect size of \(\delta = 0.15\) was used. The total sample size required for showing a significant difference between the slopes \(S_t\) and \(S_f\) was \(n = 642\) isolates for each data type (field and travellers data) per country.

**Logistic regression.** For the molecular analysis, a logistic regression model with time as a linear covariate was fitted to the prevalence of the mutant isolates (separately, for the \(pfcrt\) 76 and \(pfdhfr\) 108 data) for the travellers and field studies, for each country. Given the probability of the mutant isolates, the observed number of mutant isolates in each year was assumed to be binomially distributed. The estimated slope of the fitted logistic regression curve for the travellers and field data, the 95% confidence intervals for the slopes and whether the slopes differ significantly from each other are presented (see figures in the results section).

For the *in vitro* susceptibility analysis, a Generalized Linear Model (GLM) with a log-link function was fitted to the travellers and field data for the period 2000–2011. The slopes of the changes in CQ susceptibility for the two datasets were assessed to determine whether they differed significantly from null (0) and whether they differed significantly from each other.

**Software.** All statistical analyses were performed using Stata version 11 for Windows (Stata Corp, College Station, TX, USA) and R version 2.10 (R – project).

**Results**

Four African countries had sufficient numbers of field and traveller derived isolates to allow meaningful comparisons between the two populations: Senegal, Mali, Cote d’Ivoire and Cameroon. The characteristics for the field studies are summarised in Table 2 for each publication. A total of 23 studies were included for analysis over the 2000–2011 period, mainly from Senegal; 21 studies for \(pfdhfr\) 108 analysis for 1996–2010 and 8 studies from Senegal for the *in vitro* analysis over the same period. The characteristics of the patients differed between studies regarding the population age and the study settings (urban or rural area) but this heterogeneity was observed for the four countries.

The median patient age for travellers experiencing malaria after their return to France was 31 years, with 79% older than 15 and 61% of the travellers had visited friends and relatives in endemic countries for more than one month. Only 38% reported prophylaxis intake during their travel and most patients presented with uncomplicated malaria (95%) (Table 3). No differences among these characteristics were observed among the four countries except for gender; a majority of travellers to Senegal and Mali were male.

Between 2000–2011, 2,874 *P. falciparum* positive isolates were collected from travellers for analysis of the *pfcrt* 76 allele prevalence and 3,351 isolates for analysis of the *pfdhfr* 108 allele prevalence between 1996–2011. Between 1996 and 2011, 305 fresh blood samples were collected from travellers, and tested in Paris or Marseille to measure susceptibility to CQ *in vitro*.

Figure 2 and Table 4 summarize the temporal trends in the prevalence of the *pfcrt* 76T mutant isolates (associated with CQ susceptibility test).
resistance) in each of the targeted countries. The prevalence of the pfcrt 76T mutant genotype significantly decreased for travellers between 2000 and 2011 in Senegal (S = 2.0.17, p = 0.103), Cote d’Ivoire (S = 2.0.15, p = 0.21) and less dramatically in Cameroon (S = 2.0.09, p = 0.32). However, over that same period, no overall decrease was observed in isolates from Mali (S = 2.0.01, p = 0.72).

After comparing the slopes of the trends for the isolates from travellers (Sd) and from locally studied parasites (Sf), no significant differences were observed between 2000 and 2011 in Senegal (Sd = 2.0.17 versus Sf = 2.0.21, p = 0.58), Mali (Sd = 2.0.01 versus Sf = 2.0.01, p = 0.39), Cote d’Ivoire (Sd = 2.0.15 versus Sf = 2.0.22, p = 0.58) and Cameroon (Sd = 2.0.09 versus Sf = 2.0.05, p = 0.26) (Table 4, Figure 2). After performing a power calculation on the

Table 3. Characteristics of travellers with malaria returning from Senegal, Mali, Cote d’Ivoire and Cameroon and reported in France during the period from 2000 to 2011.

| Travellers        | Senegal (n = 1,993)* | Mali (n = 2,372)* | Cote d’Ivoire (n = 4,778 )* | Cameroon (n = 3,272)* |
|-------------------|----------------------|-------------------|-----------------------------|-----------------------|
| Median age (year) [Min-Max] | 30 [0–94]           | 31 [0–76]         | 30 [0–83]                   | 33 [0–87]             |
| Gender ratio (Male/Female) | 2.47                 | 2.20              | 1.40                        | 1.15                  |
| Chemoprophylaxis | Yes n (%)            | 746 (38)          | 959 (41)                    | 1,955 (41)            |
|                   |                      | 1,048 (32)        |
| Duration of stay  | ≤2 weeks n (%)       | 218 (13)          | 152 (8)                     | 457 (12)              |
|                   | 2–4 weeks n (%)      | 356 (21)          | 361 (18)                    | 1,150 (30)            |
|                   | 1–3 months n (%)     | 699 (41)          | 928 (48)                    | 1,221 (32)            |
|                   | >3 months n (%)      | 428 (25)          | 498 (26)                    | 1,021 (26)            |
| Purpose of travel | Tourism n (%)        | 322 (18)          | 251 (12)                    | 514 (12)              |
|                   | Visit friends and relatives n (%) | 1,108 (61) | 1,520 (71) | 2,482 (58) |
|                   | 1,738 (59)           |
| Severe malaria** | Yes n (%)            | 136 (7)           | 123 (5)                     | 225 (5)               |
|                   |                      | 177 (5)           |

*Numbers may not add to totals because of missing information.
**Severe malaria are cases of imported malaria that fulfilled at least one criteria of the WHO clinical and laboratory classification of severity [78].
doi:10.1371/journal.pone.0077775.t003

Figure 2. Observed data, fitted model (by logistic regression) and 95% confidence interval (shaded area) for the prevalence of the pfcrt 76T mutant isolates from 2000 to 2011 for travellers (red) and field studies (blue) for A-Senegal, B-Mali, C-Cote d’Ivoire and D-Cameroon. Each data point represents the prevalence of resistant isolates per year for travellers’ data and per study for field studies, where the size of the circle is proportional to the number of isolates in the sample.
doi:10.1371/journal.pone.0077775.g002
four previous tests, the probabilities of rejecting the null hypothesis, $S_t = S_0$ when it is false, were between 92 and 96%. These data derived from studies of parasites from returning travellers reflected accurately the trends of the prevalence of molecular markers of CQ resistance that were occurring in the countries in which the travellers acquired their malaria.

Changes in CQ susceptibility were also assessed using the in vitro response of isolates. From 1996 to 2011 the geometric mean of the IC$_{50}$ for CQ of the isolates tested in vitro decreased in isolates from travellers and those studied in Senegal (Table 4, Figure 3). The geometric means of the IC$_{50}$ values measured for the isolates from travellers were lower than those measured in Senegal. However, the slopes showing the trends did not differ significantly ($S_t = -0.05$ versus $S_f = -0.03$, $p = 0.26$) with a power of 94%. In this case, as well, the data gathered from travellers was an accurate reflection of the trend among parasite populations in the country of origin.

The increase of the molecular marker pfdhfr 108N has been commonly associated with an increase of pyrimethamine resistance for more than fifteen years [23]. When this parameter was compared between travellers and field-derived isolates, a significant increase in the pfdhfr 108N genotype was observed in all 4 countries over the period from 1996–2011: Senegal ($S_t = 0.12$, $p<10^{-5}$), Mali ($S_t = 0.18$, $p<10^{-5}$), Cote d’Ivoire ($S_t = 0.08$, $p<10^{-3}$) and Cameroon ($S_t = 0.21$, $p<10^{-5}$) (Table 4, Figure 4). For this comparison as well, no significant difference was observed in the trends of the molecular marker pfdhfr 108N when data from travellers ($S_t$) and field-derived ($S_f$) isolates were compared for samples taken between 1996 and 2011: Senegal ($S_t = 0.12$ versus $S_f = 0.13$, $p = 0.39$), Mali ($S_t = 0.18$ versus $S_f = 0.12$, $p = 0.12$), Cote d’Ivoire ($S_t = 0.08$ versus $S_f = 0.13$, $p = 0.48$) and Cameroon ($S_t = 0.21$ versus $S_f = 0.13$, $p = 0.75$) (Table 4, Figure 3). The powers of the four comparative analyses ranged between 91 and 97%.

Thus, all three measures of changes in prevalence of molecular markers and in vitro parasites resistance to CQ and pyrimethamine demonstrate that information from parasites imported by travellers was an accurate measure of the changes in parasites within the 4 countries studied.

### Discussion

This study suggests that the surveillance of travellers may be used for monitoring antimalarial drug resistance in endemic countries. The proof of concept was demonstrated using the prevalence of two molecular markers, pfcrt 76 and pfdhfr 108, and in vitro susceptibility for CQ. In this study, no significant difference between the trends of antimalarial drug resistance for travellers’ and field data were observed over more than 10 years. A decrease of the prevalence of the pfcrt 76T mutant genotype was observed over a period of 10 years in travellers returning from Senegal, Cote d’Ivoire and Cameroon, whilst this prevalence remains stable in Mali. An increase of mutant genotype isolates for pfdhfr 108 was observed in the four countries of West and Central Africa. The in vitro CQ susceptibility results supported the molecular results for Senegal. The trend in pfcrt 76 is downward while the trend in pfdhfr 108 is upward. The fact that screening travellers was able to detect temporal trends in opposite directions strengthens the proof of concept significantly.

Sustainable, reliable and systematic monitoring of drug efficacy is needed for tracking resistance [24]. Monitoring antimalarial drug resistance is based on clinical assessment and biological assays as part of a clinical trial [25]. Since the emergence of resistance to CQ, and then later to SP, capacities to conduct such monitoring in endemic countries have substantially improved, but remain very heterogeneous. In particular regions, human and/or technical resources are limited and, as such, conducting a clinical trial for the purpose of surveillance has competed with other high priorities.

### Table 4. Comparison between travellers and field data for the pfcrt 76 and pfdhfr 108 molecular markers and for the CQ in vitro susceptibility in Senegal.

| Country | Travellers Slope [95% CI*] | Field Study Slope [95% CI] | p-value** |
|---------|----------------------------|----------------------------|-----------|
| **Pfcrt 76** | | | |
| Senegal | $-0.167$ [−0.219; $-0.115$] | $-0.208$ [−0.312; $-0.105$] | 0.575 |
| Mali | $-0.009$ [−0.082; 0.063] | 0.005 [−0.106; 0.116] | 0.885 |
| Cote d’Ivoire | $-0.146$ [−0.215; $-0.078$] | $-0.215$ [−0.463; 0.032] | 0.578 |
| Cameroon | $-0.090$ [−0.146; $-0.033$] | 0.050 [−0.220; 0.321] | 0.264 |
| **Pfdhfr 108** | | | |
| Senegal | 0.117 [0.088; 0.147] | 0.148 [0.088; 0.209] | 0.386 |
| Mali | 0.182 [0.124; 0.240] | 0.119 [0.086; 0.152] | 0.116 |
| Cote d’Ivoire | 0.083 [0.052; 0.113] | 0.132 [−0.025; 0.289] | 0.484 |
| Cameroon | 0.213 [0.115; 0.311] | 0.130 [−0.155; 0.415] | 0.753 |
| **CQ in vitro analysis** | | | |
| Senegal | $-0.050$ [−0.085; $-0.015$] | $-0.028$ [−0.059; 0.002] | 0.264 |

*CI = confidence interval, **The p-value indicates whether the fitted slopes for travellers data and field studies were significantly different from each other.

doi:10.1371/journal.pone.0077775.t004

Figure 3. Observed data, fitted model (by Generalized Linear Model) and 95% confidence interval (shaded area) for the in vitro CQ response (IC$_{50}$) isolates from 1996 to 2011 for travellers (red) and field studies (blue) from Senegal. Each data point represents the ln (mean IC$_{50}$ per year for travellers’ data and per study for field studies, where the size of the circle is proportional to the number of isolates in the sample.

doi:10.1371/journal.pone.0077775.g003

Figure 3. Observed data, fitted model (by Generalized Linear Model) and 95% confidence interval (shaded area) for the in vitro CQ response (IC$_{50}$) isolates from 1996 to 2011 for travellers (red) and field studies (blue) from Senegal. Each data point represents the ln (mean IC$_{50}$ per year for travellers’ data and per study for field studies, where the size of the circle is proportional to the number of isolates in the sample.

doi:10.1371/journal.pone.0077775.g003

Table 4. Comparison between travellers and field data for the pfcrt 76 and pfdhfr 108 molecular markers and for the CQ in vitro susceptibility in Senegal.

| Country | Travellers Slope [95% CI*] | Field Study Slope [95% CI] | p-value** |
|---------|----------------------------|----------------------------|-----------|
| **Pfcrt 76** | | | |
| Senegal | $-0.167$ [−0.219; $-0.115$] | $-0.208$ [−0.312; $-0.105$] | 0.575 |
| Mali | $-0.009$ [−0.082; 0.063] | 0.005 [−0.106; 0.116] | 0.885 |
| Cote d’Ivoire | $-0.146$ [−0.215; $-0.078$] | $-0.215$ [−0.463; 0.032] | 0.578 |
| Cameroon | $-0.090$ [−0.146; $-0.033$] | 0.050 [−0.220; 0.321] | 0.264 |
| **Pfdhfr 108** | | | |
| Senegal | 0.117 [0.088; 0.147] | 0.148 [0.088; 0.209] | 0.386 |
| Mali | 0.182 [0.124; 0.240] | 0.119 [0.086; 0.152] | 0.116 |
| Cote d’Ivoire | 0.083 [0.052; 0.113] | 0.132 [−0.025; 0.289] | 0.484 |
| Cameroon | 0.213 [0.115; 0.311] | 0.130 [−0.155; 0.415] | 0.753 |
| **CQ in vitro analysis** | | | |
| Senegal | $-0.050$ [−0.085; $-0.015$] | $-0.028$ [−0.059; 0.002] | 0.264 |

*CI = confidence interval, **The p-value indicates whether the fitted slopes for travellers data and field studies were significantly different from each other.

doi:10.1371/journal.pone.0077775.t004
that Ministries of Health must contend with and has not been systematically conducted.

Previous studies highlight the usefulness of travellers’ surveillance as an early warning detection system for emergence or re-emergence of communicable diseases [26,27,28]. Travellers’ surveillance has proven in the past to be an effective early alert system for detecting the emergence of CQ resistance (Table 1). One strength of using travellers as a sentinel system of resistance is that detection of clinical therapeutic failure due to resistance is facilitated in this non immune population with a low risk of re-infection. Moreover, the French Malaria Reference Centre use standardized methods for prospectively collecting reliable information.

This study does have several limitations. First, due to the complexity of collecting laboratory data systematically, consistently and over a long period of time in both populations, travellers and field studies, only four countries and two molecular markers have been used in this proof of concept. Second, precise information regarding the location of infection within each country could not be collected for *P. falciparum* infected travellers returning from endemic countries. However, travellers did not visit all parts of a country and they were more likely to frequent particular places such as touristic and/or, or business-oriented locations. The reported information was highly dependent on factors such as the areas that were visited, the period of travel, migration history and the political context in endemic areas. Of course, these factors can also impact information on exact locations where patients acquire their infections within the country, as well. Perhaps more importantly, the travellers in this work were not representative of the native population in that their baseline characteristics differ, including age, immune status and parasitemia before treatment. Finally, especially for the field studies, different approaches were used for determining the molecular markers of resistance and the *in vitro* susceptibility for CQ. The heterogeneity between methods is encouraging WWARN to standardise approaches and to develop common tools like IVART [21].

However, these limitations do not diminish the clarity of the outcome presented here. Surveillance of parasites from travellers provided an accurate picture of events occurring in the field. This does not suggest that this approach should replace studies conducted in endemic countries. Rather, information from travellers can be used as an additional surveillance system.

Given the utility, surveillance of travellers can be useful in tracking resistance to ACTs, as well. Currently, only the response to the long-acting partner drugs, can be assessed, but if putative molecular markers are defined, tracking of resistance to the artemisinin component can also be added. The collaboration between Ministries of Health in endemic countries and the malaria reference centres in non-endemic countries for sharing and validating collected information should be reinforced and facilitated.

Due to the length of time between a field study and the publication of results, data collected from imported cases may be

![Observed data, fitted model (by logistic regression) and 95% confidence interval (shaded area) for the prevalence of the pfHFr-108 mutant isolates from 1996 to 2011 for travellers (red) and field studies (blue) for A-Senegal, B-Mali, C-Cote d'Ivoire and D-Cameroon.](https://doi.org/10.1371/journal.pone.0077775.g004)

Figure 4. Observed data, fitted model (by logistic regression) and 95% confidence interval (shaded area) for the prevalence of the *pfHFr*-108 mutant isolates from 1996 to 2011 for travellers (red) and field studies (blue) for A-Senegal, B-Mali, C-Cote d'Ivoire and D-Cameroon. Each data point represents the prevalence of resistant isolates per year for travellers’ data and per study for field studies, where the size of the circle is proportional to the number of isolates in the sample. doi:10.1371/journal.pone.0077775.g004
available in a more timely manner and, as such, could be used for early alert of emerging resistance. The complexity of the available tools for assessing drug efficacy and monitoring resistance highlights the importance of a standardized and coordinated approach. The follow-up of imported cases in several non-endemic countries should also enable the collaborators to track the evolution of resistance to antimalarial drugs at an international scale and thus provide novel information of value to policy makers.

The goal of this work was to validate the use of international traveller surveillance systems, for detecting the emergence of antimalarial drug resistance and for following resistance trends where local information is not otherwise available and/or sufficient. Easy access to reproducible and standardized data should be implemented. The existing health international, European or American institutions (WHO, European Centre for Disease Prevention and Control (ECDC), US Centres for Disease Control and Prevention (CDC Atlanta) and the different networks for infectious diseases surveillance in travellers (TropNet Europe, EuroTravNet, GeoSentinel) should be used for facilitating the coordination and data sharing between national surveillance systems [26,27,28].

Conclusions

This study has not shown different trends in antimalarial drug resistance between travellers and field studies. An international travellers’ database can be used as an additional surveillance system to assess and monitor the emergence of drug resistance in endemic areas where information is limited.

Acknowledgments

We thank Prof Carol Sibley for critical reading of the manuscript. The authors would like to thank Sandie Menard and Antoine Berry for generously providing additional information and data from their previous publication. The authors would like to thank Fabrice Legros (deceased) for his contribution to the study.

This article has been submitted on behalf of the National Malaria Reference Centre Study Group

Ahmed Aboubacar (Institut de parasitologie et de pathologie tropicale, Strasbourg, France), Patrice Agnamey (Service de Parasitologie, CHU Amiens, France), Adela Angoulvant (Service de Parasitologie, APHP Bicêtre, Paris, France), Patricia Barbut (Laboratoire de Biologie Médicale, CH Longjumeau, France), Didier Basset (Service Parasitologie, CHU Montpellier, France), Ghania Belkadi (Service Parasitologie, APHP Saint Antoine, Paris, France), Anne-Pauline Bellanger (Service Parasitologie, CHRU Jean Minjoz, Besançon, France), Dieudonné Bemba (Service Microbiologie, APHP Bondy-Jeande Verdier, Paris, France), Françoise Benoît-Vical (Service Parasitologie, CHU Rangueil, Toulouse, France), Antoine Berry (Service de Parasitologie, CHU Rangueil, Toulouse, France), Marie-Laure Bigel (Laboratoire de Biologie Médicale, CHU Creil, France), Laurent Bret (Service Microbiologie, CHR Créteil, France), Pierre Buffet (Service Parasitologie, CHU Caen, France), Françoise Botrel (Service de Parasitologie, APHP Henri Mondor, Créteil, France), Olivier Bouchaud (Service de Maladies Infectieuses et Tropicales, CHU Avicenne-Bobigny, Paris, France), Marie-Élisa Boignoux (Service Microbiologie, APHP Necker, Paris, France), Pascal Delaunay (Service Parasitologie, CHU Limoges, France), Ludovic De Gentile (Service de médecine tropicales, CHU Angers, France), Jean-Marie Delarbre (Service Microbiologie, CH Mulhouse, France), Pascal Delaunay (Service Parasitologie, CHU Nice, France), Anne Delaval (Service Microbiologie, CHG Aulney-sous-Bois, France), Jean Delmont (Service Médecine Tropicale et Infectieuse, Hôpital Nord, Marseille, France), Guillaume Desoubeaux (Service Parasitologie, CHRU de Tours, France), Michel Devleux (Service Parasitologie, APHP Saint Antoine, Paris, France), Jean Dunand (Service Microbiologie et Hygiène, APHP Ambroise Paré, Paris, France), Rémy Durand (Service Parasitologie, APHP Avicenne-Bobigny, Paris, France), Odile Eloy (Service Parasitologie, CH Versailles, France), Nathalie Fauchet (Service Microbiologie, CHU Créteil, France), Bernard Fauge (Service Parasitologie, APHM Timone, Marseille, France), Albert Faye (Service Pédiatrie, APHP Robert Debré, Paris, France), Pierre Flori (Service Parasitologie, CHU Saint-Etienne, France), Chantal Garabedian (Laboratoire de Maladies Infectieuses et Hygiène, CH Pays d’Aix, Aix-en-Provence, France), Françoise Gay-Audruie (Service Parasitologie, CHU Nantes, France), Nadine Godineau (Service Microbiologie, CHG Delafontaine, St Denis, France), Pascal Houzé (Laboratoire Biochimie, APHP Saint Louis, Paris, France), Sandrine Houzé (Service Parasitologie, APHP Bichat-Claude Bernard, Paris, France), Houria Ichou (Service Microbiologie et Hygiène, APHP Louis Mourier, Paris, France), Laurence Lachauch (Service Microbiologie, CHU Nimes, France), Magalie Lecerv (Laboratoire de Biologie Médicale, CH Civil, France), Gwendal Le Moal (Service Maladies Infectieuses, CHU Poitiers, France), Marie Machonart (Service Parasitologie, CHU Nancy, France), Denis Malvy (Service Parasitologie, CHU Bordeaux, France), Sophie Matheron (Service Maladies Infectieuses et Tropicales, APHP Bichat-Claude Bernard, Paris, France), Danièle Maunou (Service Parasitologie, CHU Grenoble, France), Bruno Megarbane (Service de Réanimation Médicale et Toxicologie, APHP Lariboisière, Paris, France), Sandie Menard (Service de Parasitologie, CHU Rangueil, Toulouse, France), Laurence Millon (Service Parasitologie, CHRU Jean Minjoz, Besançon, France), Muriel Minoum-Aiach (Service Microbiologie, APHP Trousseau, Paris, France), Philippe Minodier (Service Pédiatrie, APHM Hôpital Nord, Marseille, France), Gilles Neveu (Service Parasitologie, CHU Brest, France), Philippe Parola (Service Maladies Infectieuses et Tropicales APHM Hôpital Nord, Marseille, France), Daniel Parzy (Service Parasitologie, IMTSSA, Marseille, France), Olivier Patey (Service Maladies Infectieuses et Tropicales, CHU Villeneuve Saint Georges, France), Pierre Patoz (Laboratoire Biologie Médicale, CH Tourcoing, France), Pascale Penn (Service Microbiologie, CH Le Mans, France), Alice Perignon (Service de maladies infectieuses et tropicales, APHP Pitie-Salpétrière, Paris, France), Stéphane Picot (Service Parasitologie, CHU Lyon, France), Jean-Etienne Pilo (Service de Santé et des Armées Maladies Infectieuses et Tropicales, HIA Bégin, Saint Mandé, France), Isabelle Poilane (Service Microbiologie, APHP Bondy Jean Vilar, Paris, France), Pierre Pons (Service Parasitologie, CHU Clermont Ferrand, France), Christophe Rapp (Service de Santé et des Armées Maladies Infectieuses et Tropicales, HIA Bégin, Saint Mandé, France), Marie-Catherine Receveur (Service Médecine, CHU Bordeaux, France), Claudine Sarfati (Service Parasitologie, APHP Saint-Louis Lariboisière, Paris, France), Yaye Senghor (Service Parasitologie, APHP Avicenne, Paris, France), Jean-Yves Siriez (Service Pédiatrie, APHP Robert Debré, Paris, France), Nicolas Taudon (Service Parasitologie, IMTSSA, Marseille, France), Marc Thellier (Service Parasitologie, APHP Pitie-Salpêtrière, Paris, France), Maxime Thouvenin (Service Microbiologie, CH Troye, France), Dominique Toubas (Service Parasitologie, CHU Reims, France).

Author Contributions

Conceived and designed the experiments: MG PJG JL. Performed the experiments: MG JAF BP AB MN AAD VH PB OG ATO LP. Analyzed the data: MG PJG JL. Wrote the paper: MG PJG JL.

References

1. Phyo AP, Nkoma S, Stepanyukova K, Ashley EA, Nair S, et al. (2012) Emergence of artemisinin-resistant malaria on the western border of Thailand: a longitudinal study. Lancet 379: 1960–1966.

2. Dondorp AM, Nosten F, Yi P, Das D, Phyo AP, et al. (2009) Artemisinin resistance in Plasmodium falciparum malaria. N Engl J Med 361: 455–467.

PLOS ONE | www.plosone.org 9 October 2013 | Volume 8 | Issue 10 | e77775
Le Bras J, Coulaud JP, Briceira F, Le Bras M, Roue R, et al. (1985) Chloroquine-resistant falciparum malaria in the Congo. Lancet 2: 1071.

Sanoussi P, Lebras C, Verdier F, Charmot G, Dupont B, et al. (1985) Chloroquine-resistant Plasmodium falciparum in Cameroon. Lancet 1: 1134–1135.

Ölsen VV, Jensen T, Jorgensen M (1984) Chloroquine-resistant Plasmodium falciparum malaria from Angola. Lancet 1: 1462–1463.

Neequeyre J, Coene J, Taelman H, Weyl M, Greenberg AE, et al. (1986) In vivo chloroquine-resistant falciparum malaria in western Africa. Lancet 1: 2.

Le Bras J, Hatin I, Bourre P, Coco-Cianci O, Garin JP, et al. (1986) Chloroquine-resistant falciparum malaria in Benin. Lancet 2: 1043–1044.

Hellgren U, Arthil OK, Leibard M, Rombo L (1987) Is chloroquine-resistant Plasmodium falciparum malaria emerging in Senegal or the Gambia? Trans R Soc Trop Med Hyg 81: 721.

Charmot G, Le Bras J, Doury JC, Baudoin D, Roue R, et al. (1988) Chloroquine-resistant Plasmodium falciparum malaria from Ivory Coast. Trans R Soc Trop Med Hyg 82: 392–393.

Chabasse D, De Gentile L, Laggy C, Le Bras J, Riadlad X, et al. (1988) Chloroquine-resistant Plasmodium falciparum malaria in Mali revealed by congenital malaria. Trans R Soc Trop Med Hyg 82: 541.

Thomas SM, Ndir O, Diouf T, Mboup S, Wypij D, et al. (2005) In vitro chloroquine susceptibility and PCR analysis of pfcr and pfmdr1 polymorphisms in Plasmodium falciparum isolates from Senegal. Am J Trop Med Hyg 66: 474–480.

Sarr O, Myrick A, Daily J, Diop RM, Dieng T, et al. (2005) In vivo and in vitro analysis of chloroquine resistance in Plasmodium falciparum isolates from Senegal. Parasitol Res 97: 136–140.

Bertin G, Ndam NT, Fajuri-Jemoursi S, Fievet N, Renant E, et al. (2005) High prevalence of Plasmodium falciparum K76T mutation in pregnant women taking chloroquine prophylaxis in Senegal. J Antimicrob Chemother 50: 778–781.

Henry M, Dielio I, Bordes J, Ka S, Pradines B, et al. (2006) Urban malaria in Dakar, Senegal: chemosusceptibility and genetic diversity of Plasmodium falciparum isolates. Am J Trop Med Hyg 75: 146–151.

Sarr O, Ahouadi AD, Ly O, Daily J, Ndiaye D, et al. (2008) Mutations in pfcr K76T do not correlate with sulfadoxine-pyrimethamine-amodiaquine failure in P. falciparum isolates from Senegal. J Exp Ther Res 103: 765–769.

Bob NS, Diop BM, Renard A, Marra LA, Durand P, et al. (2010) Parasite polymorphism and severe malaria in Dakar (Senegal): a West African urban area. PloS One 5: e9817.

Ndiaye D, Patel V, Demas A, LeRoux M, Nelir O, et al. (2010) A non-radioactive DAPI-based high-throughput in vitro assay to assess Plasmodium falciparum responsiveness to antimalarial-increased sensitivity of P. falciparum to chloroquine in Senegal. Am J Trop Med Hyg 82: 226–230.

Wurz N, Fall B, Pascual A, Diascara S, Sow K, et al. (2012) Prevalence of molecular markers of Plasmodium falciparum drug resistance in Dakar, Senegal. Malar J 11: 197.

Ndiaye M, Faye B, Tine R, L’aye JL, Lo A, et al. (2012) Assessment of the Molecular Markers of Plasmodium falciparum Chloroquine Resistance (Pfcrt) in Senegal after Several Years of Chloroquine Withdrawal. Am J Trop Med Hyg 87: 640–645.

Tekete M, Djeem AA, Beavogui AH, Maiga H, Sagara I, et al. (2009) Efficacy of sulfadoxine/pyrimethamine/amodiaquine failure in P. falciparum isolates from Khémis, Senegal. Plasmodo 185: 380–389.

Bob NS, Diop BM, Renaud A, Marra LA, Durand P, et al. (2010) Parasite polymorphism and severe malaria in Dakar (Senegal): a West African urban area. PloS One 5: e9817.

Ndiaye D, Patel V, Demas A, LeRoux M, Nelir O, et al. (2010) A non-radioactive DAPI-based high-throughput in vitro assay to assess Plasmodium falciparum responsiveness to antimalarial-increased sensitivity of P. falciparum to chloroquine in Senegal. Am J Trop Med Hyg 82: 226–230.

Wurz N, Fall B, Pascual A, Diascara S, Sow K, et al. (2012) Prevalence of molecular markers of Plasmodium falciparum drug resistance in Dakar, Senegal. Malar J 11: 197.

Wele M, Djeem AA, Guindo A, Beavogui AH, Traoré IZ, et al. (2012) High prevalence of P. falciparum K76T mutation in pregnant women taking chloroquine prophylaxis in Senegal. J Exp Ther Res 103: 765–769.

Bob NS, Diop BM, Renard A, Marra LA, Durand P, et al. (2010) Parasite polymorphism and severe malaria in Dakar (Senegal): a West African urban area. PloS One 5: e9817.

Ndiaye D, Patel V, Demas A, LeRoux M, Nelir O, et al. (2010) A non-radioactive DAPI-based high-throughput in vitro assay to assess Plasmodium falciparum responsiveness to antimalarial-increased sensitivity of P. falciparum to chloroquine in Senegal. Am J Trop Med Hyg 82: 226–230.

Wurz N, Fall B, Pascual A, Diascara S, Sow K, et al. (2012) Prevalence of molecular markers of Plasmodium falciparum drug resistance in Dakar, Senegal. Malar J 11: 197.

Ndiaye M, Faye B, Tine R, L’aye JL, Lo A, et al. (2012) Assessment of the Molecular Markers of Plasmodium falciparum Chloroquine Resistance (Pfcrt) in Senegal after Several Years of Chloroquine Withdrawal. Am J Trop Med Hyg 87: 640–645.

Tekete M, Djeem AA, Beavogui AH, Maiga H, Sagara I, et al. (2009) Efficacy of sulfadoxine/pyrimethamine/amodiaquine failure in P. falciparum isolates from Khémis, Senegal. Plasmodo 185: 380–389.

Bob NS, Diop BM, Renard A, Marra LA, Durand P, et al. (2010) Parasite polymorphism and severe malaria in Dakar (Senegal): a West African urban area. PloS One 5: e9817.
59. Mbacham WF, Evehe MS, Netongo PM, Ateh IA, Mimche PN, et al. (2010) Efficacy of amodiaquine, sulphadoxine-pyrimethamine and their combination for the treatment of uncomplicated Plasmodium falciparum malaria in children in Cameroon at the time of policy change to artemisinin-based combination therapy. Malar J 9: 34.

60. Menard S, Morlais I, Tahar R, Sayang C, Manyungu PL, et al. (2012) Molecular monitoring of Plasmodium falciparum drug susceptibility at the time of the introduction of artemisinin-based combination therapy in Yaoundé, Cameroon: implications for the future. Malar J 11: 113.

61. Noronate N, Durand R, Tall A, Marrama L, Spiegel A, et al. (2007) Rapid dissemination of Plasmodium falciparum drug resistance despite strictly controlled antimalarial use. PLAb One 2: e139.

62. NDiaye D, Daily JP, Sarr O, Ndé O, Gaye O, et al. (2003) Mutations in Plasmodium falciparum dihydrofolate reductase and dihydropteroate synthase genes in Senegal. Trop Med Int Health 10: 1176–1179.

63. Faye B, NDiaye M, NDiaye JL, Annie A, Tine RC, et al. (2011) Prevalence of molecular markers of Plasmodium falciparum resistance to sulfadoxine-pyrimethamine during the intermittent preventive treatment in infants coupled with the expanded program immunization in Senegal. Parasitol Res 109: 133–139.

64. Doumbo OK, Kayentao K, Djimde A, Cortese JF, Diouf Y, et al. (2000) Rapid selection of Plasmodium falciparum dihydrofolate reductase mutants by pyrimethamine prophylaxis. J Infect Dis 182: 993–996.

65. Djimde AA, Dolo A, Ouattara A, Diakité S, Fofou CV, et al. (2004) Molecular diagnosis of resistance to antimalarial drugs during epidemics and in war zones. J Infect Dis 190: 453–455.

66. Thera MA, Selelve PS, Coulibaly D, Traoré K, Garba MN, et al. (2005) Impact of trimethoprim-sulfamethoxazole prophylaxis on falciparum malaria infection and disease. J Infect Dis 192: 1823–1829.

67. Dicko A, Sagra I, Djimde AA, Toure SO, Traoré M, et al. (2010) Molecular markers of resistance to sulfadoxine-pyrimethamine one year after implementation of intermittent preventive treatment in infants in Mali. Malar J 9: 9.

68. Djaman JA, Mazabraud A, Basco L (2007) Sulphadoxine-pyrimethamine susceptibilities and analysis of the dihydrofolate reductase and dihydropteroate synthase of Plasmodium falciparum isolates from Côte d’Ivoire. Ann Trop Med Parasitol 101: 103–112.

69. Basco LK, Ndongu M, Tejiokem M, Ngane VF, Youmba JC, et al. (2002) Molecular epidemiology of malaria in Cameroon. XI. Geographic distribution of Plasmodium falciparum isolates with dihydrofolate reductase gene mutations in southern and central Cameroon. Ann Trop Med Hyg 67: 378–382.

70. Tahar R, Basco LK (2000) Molecular epidemiology of malaria in Cameroon. XXII. Geographic mapping and distribution of Plasmodium falciparum dihydrofolate reductase (dhfr) mutant alleles. Ann Trop Med Hyg 75: 396–401.

71. Mbacham WF, Evehe MSB, Netongo PM, Ali IM, Nfor NE, et al. (2009) Mutations within folate metabolising genes of Plasmodium falciparum in Cameroon. African Journal of Biotechnology 8: 6.

72. McCollum AM, Basco LK, Tahar R, Udhayakumar V, Escalante AA (2008) Hitchhiking and selective sweeps of Plasmodium falciparum sulfadoxine and pyrimethamine resistance alleles in a population from central Africa. Antimicrobial Agents Chemotherapy 52: 4089–4097.

73. Brassier P, Guiguermele R, Daibo S, Guiyedi V, Kombila M, et al. (1999) Amodiaquine remains effective for treating uncomplicated malaria in west and central Africa. Trans R Soc Trop Med Hyg 93: 645–650.

74. Pradines B, Tall A, Parzy D, Spiegel A, Faisst T, et al. (1998) In-vitro activity of pyronaridine and amodiaquine against African isolates (Senegal) of Plasmodium falciparum in comparison with standard antimalarial agents. J Antimicrob Chemother 42: 333–339.

75. Pradines B, Tall A, Rogier C, Spiegel A, Monier J, et al. (2002) In vitro activities of ferrochloroquine against 53 Senegalese isolates of Plasmodium falciparum in comparison with those of standard antimalarial drugs. Trop Med Int Health 7: 265–270.

76. Pradines B, Tall A, Ramondrasoa F, Spiegel A, Sokhna C, et al. (2006) In vitro activity of iron-binding compounds against Senegalese isolates of Plasmodium falciparum. J Antimicrob Chemother 57: 1093–1099.

77. Fall B, Diawara S, Sow K, Baret E, Diatta B, et al. (2011) Ex vivo susceptibility of Plasmodium falciparum isolates from Dakar, Senegal, to seven standard antimalarial drugs. Malar J 10: 310.

78. WHO (2013) Management of severe malaria: A practical handbook. Geneva: World Health Organization. 90 p.