Prevalence of In Vitro Resistance to Eleven Standard or New Antimalarial Drugs among *Plasmodium falciparum* Isolates from Pointe-Noire, Republic of the Congo

Bruno Pradines,^{1,2*} Philippe Hovette,³ Thierry Fusai,^{1,2} Henri Léonard Atanda,³ Eric Baret,^{1,2} Philippe Cheval,³ Joel Mosnier,^{1,2} Alain Callec,³ Julien Cren,^{4,2} Rémy Amalvict,^{1,2} Jean Pierre Gardair,⁵ and Christophe Rogier^{1,2}

Unité de Recherche en Biologie et Epidémiologie Parasitaires, Institut de Médecine Tropicale du Service de Santé des Armées, Le Pharo, 13998 Marseille, France¹; Institut Fédératif de la Recherche no. 48, 13385 Marseille, France²; Centre Médical de Secours, Total Exploration et Production Congo, Pointe-Noire, Republic of the Congo³; Unité de Recherche en Physiologie et Pharmacocinétique Parasitaires, Institut de Médecine Tropicale du Service de Santé des Armées, Le Pharo, 13998 Marseille, France⁴; and Département Médical International, Total, 92078 Paris la Défense, France⁵

Received 23 March 2006/Accepted 11 May 2006

We determined the level of in vitro resistance of *Plasmodium falciparum* parasites to standard antimalarial drugs, such as chloroquine, quinine, amodiaquine, halofantrine, mefloquine, cycloguanil, and pyrimethamine, and to new compounds, such as dihydroartemisinin, doxycycline, atovaquone, and lumefantrine. The in vitro resistance to chloroquine reached 75.5%. Twenty-eight percent of the isolates were intermediate or had reduced susceptibility to quinine. Seventy-six percent and 96% of the tested isolates showed in vitro resistance or intermediate susceptibilities to cycloguanil and pyrimethamine, respectively. Only 2% of the parasites demonstrated in vitro resistance to monodesethylamodiaquine. No resistance was shown with halofantrine, lumefantrine, dihydroartemisinin, or atovaquone. Halofantrine, mefloquine, and lumefantrine demonstrated high correlation. No cross-resistance was identified between responses to monodesethyl-amodiaquine, dihydroartemisinin, atovaquone, and cycloguanil. Since the level of chloroquine resistance in vitro exceed an unacceptable upper limit, high rates of in vitro resistance to pyrimethamine and cycloguanil and diminution of the susceptibility to quinine, antimalarial drugs used in combination, such as amodiaquine, artemisinin derivatives, mefloquine, lumefantrine, or atovaquone, seem to be appropriate alternatives for the first line of treatment of acute, uncomplicated *P. falciparum* malaria.

In the Republic of the Congo, malaria-attributable morbidity and mortality in children constitutes a major public health problem (11, 20). Rational policies for malaria therapy are the primary tools for responding to this reemerging disease. If most countries south of the equator have already replaced chloroquine as the first-line therapy for uncomplicated malaria, chloroquine is still the first-line drug for treatment of malaria in the Republic of the Congo (36, 37), where malaria is endemic. The first reports of chloroquine resistance in Zaïre and in the Republic of the Congo (Pointe-Noire and Brazzaville) were in 1984 and 1985, respectively (19, 25). Since then, the chloroquine treatment failure rate has rapidly increased, from 39% in 1986 (10) to 52% in 1992 (7) and 62.5% in 2002 (26) in Brazzaville. In Pointe-Noire, in vivo chloroquine resistance increased from 25% in 1986 (34) to 43% in 1999 (26). The emergence and spread of amodiaquine resistance was also demonstrated: 29% in 1986 (10) versus 43% in 1996 (14). The emergence of halofantrine resistance was reported in 1992 in Brazzaville (8). In vitro chemosusceptibility studies conducted in Brazzaville from 1987 to 1993 showed chloroquine-resistant isolate rates ranging from 21% to 58% (7, 12, 13, 15). The assessment of chloroquine resistance by PCR and sequencing

methods (identification of mutations in a *Plasmodium falciparum* chloroquine resistance transporter gene) showed that the K76T mutation in *Pfcr* was present in 98 to 99% of isolates from Brazzaville since 1999 (22, 27). As far back as 1986, *P. falciparum* isolates in Pointe-Noire showed high prevalence of chloroquine resistance by in vitro tests (82%) (34) or the PCR method (93%) (27). Only a few data, usually with a small number of samples which does not allow a statistically meaningful analysis, are available on other antimalarial drugs, such as quinine, halofantrine, mefloquine, amodiaquine, and pyrimethamine.

As the Republic of the Congo is suffering from recurrent security problems in some regions (where the in vivo test cannot be performed as a consequence), the in vitro test in determining the risk of antimalarial drug resistance could help to provide timely and inexpensive data at the national level to make rational choices for malaria treatment policy. Increasing reports of resistance to chloroquine have created an urgent need for study of an appropriate first-line therapeutic alternative. The objectives of this study were to determine the level of resistance of *P. falciparum* parasites to standard antimalarial drugs, such as chloroquine, quinine, amodiaquine, halofantrine, mefloquine, cycloguanil, and pyrimethamine, and to new compounds, such as dihydroartemisinin, doxycycline, atovaquone, and lumefantrine. This is the first such study in the Republic of the Congo.

* Corresponding author. Mailing address: IMTSSA, URBEP, Bd. C. Livon, Parc le Pharo, BP 46, 13998 Marseille Armées, France. Phone: 33 4 91 15 01 10. Fax: 33 4 91 15 01 64. E-mail: bruno.pradines@free.fr.

TABLE 1. In vitro susceptibilities and prevalence of resistance^a

Drug	No. of isolates tested	Mean IC ₅₀	95% Confidence interval	% Resistance ^b
Chloroquine	110	141 nM	122-162 nM	75.5
Quinine	110	316 nM	280-357 nM	6 (22) a
Monodesethylamodiaquine	90	22.4 nM	20.0-24.8 nM	2
Mefloquine	110	9.3 nM	8.2-10.5 nM	7
Halofantrine	40	0.79 nM	0.62-1.02 nM	0
Lumefantrine	91	6.8 nM	5.7-8.2 nM	0
Dihydroartemisinin	110	1.02 nM	0.85-1.23 nM	0
Atovaquone	110	3.5 nM	3.0-4.1 nM	0
Doxycycline	109	8.2 µM	7.3-9.1 µM	ND
Cycloguanil	104	277 nM	222-346 nM	36 (40) b
Pyrimethamine	68	2506 nM	1938-3241 nM	68 (28) c

^a Shown are in vitro susceptibilities and prevalence of resistance or reduced susceptibility of *P. falciparum* isolates from Pointe-Noire to antimalarial drugs such as chloroquine, quinine, monodesethylamodiaquine, mefloquine, halofantrine, lumefantrine, dihydroartemisinin, atovaquone, doxycycline, cycloguanil, and pyrimethamine.

^b The cutoff values for in vitro resistance or reduced susceptibility were the following: chloroquine, 100 nM (18); quinine, 800 nM (4) ("a" indicates intermediate susceptibility between 500 and 800 nM); monodesethylamodiaquine, 60 nM (5); mefloquine, 30 nM (16); halofantrine, 6 nM (3); lumefantrine, 150 nM (32); dihydroartemisinin, 10.5 nM (33); atovaquone, 1,900 nM (24); cycloguanil, 500 nM (6) ("b" indicates intermediate susceptibility between 100 and 500 nM); and pyrimethamine, 2,000 nM (6) ("c" indicates intermediate susceptibility between 500 and 2,000 nM). The cutoff value for in vitro reduced susceptibility to doxycycline has not yet been determined (ND).

MATERIALS AND METHODS

Isolates of *Plasmodium falciparum*. Isolates of *P. falciparum* were collected from malaria patients from Pointe-Noire, the economic capital of the Republic of Congo, with 600,000 inhabitants. All patients and their parents or guardians were briefed on the project and provided verbal informed consent prior to collection of blood by venipuncture. The local health (Société Médicale du Kouilou) and institutional authorities approved the research protocol.

This study took place from March 2005 to January 2006 in the Service Médical of Total-Elf. The children, aged 16 months to 17 years, were enrolled if they presented to the health center because of febrile illness and had uncomplicated malaria. Patients were treated by halofantrine, lumefantrine-artemether, amodiaquine-artesunate, quinine, or sulfadoxine-pyrimethamine, following the recommended therapeutic protocols and dosages.

One-hundred sixty-four venous blood samples were collected before treatment in Vacutainer ACD tubes (Becton Dickinson, Rutherford, NJ) and transported at 4°C to our laboratory in Marseille, France, within less than 72 h of collection. Thin blood smears were stained using an RAL kit (Réactifs RAL, Paris, France) and examined to determine *P. falciparum* density. Parasitized erythrocytes were washed three times in RPMI 1640 medium (Invitrogen, Paisley, United Kingdom). If parasitemia exceeded 0.8%, infected erythrocytes were diluted to 0.5 to 0.8% with uninfected erythrocytes and resuspended in culture medium to a hematocrit level of 1.5%. Susceptibilities to chloroquine, quinine, monodesethylamodiaquine, mefloquine, halofantrine, lumefantrine, doxycycline, atovaquone, and dihydroartemisinin were determined after suspension in RPMI 1640 medium and to cycloguanil and pyrimethamine after suspension in RPMI 1640 SP823 with reduced *p*-aminobenzoic acid (0.5 µg/liter) and low folates (10 µg/liter) (Invitrogen). The two suspensions were supplemented with 10% human serum and buffered with 25 mM HEPES and 25 mM NaHCO₃.

Drugs. Chloroquine diphosphate, quinine hydrochloride, doxycycline hydrochloride, dihydroartemisinin, and pyrimethamine were obtained from Sigma (St. Louis, MO); atovaquone and halofantrine were obtained from GlaxoSmithKline (Evreux, France); lumefantrine was obtained from Novartis Pharma (Basel, Switzerland); mefloquine was obtained from Roche (Paris, France); cycloguanil was obtained from Zeneca Pharma (Reims, France); and monodesethylamodiaquine was obtained from the World Health Organization. Stock solutions were prepared in ethanol for lumefantrine; in methanol for mefloquine, doxycycline, atovaquone, quinine, dihydroartemisinin, monodesethylamodiaquine, and pyrimethamine; and in sterile water for primaquine, chloroquine, amodiaquine, and cycloguanil. Twofold serial dilutions were prepared in sterile water and distributed in triplicate into Falcon 96-well flat-bottomed plates (Becton Dickinson, Franklin Lakes, NJ). The chloroquine-susceptible 3D7 *P. falciparum* clone (Africa) and the chloroquine-resistant W2 clone (Indochina) were used as references to test each batch of plates. Reference clones were maintained in continuous culture and twice synchronized with sorbitol (17).

Drug assay. For in vitro isotopic microtests, 200 µl/well of the suspension of parasitized erythrocytes was distributed in 96-well plates preloaded with antimalarial agents. Parasite growth was assessed by adding 1 µCi of [³H]hypoxanthine

with a specific activity of 14.1 Ci/mmol (NEN Products, Dreiech, Germany) to each well. Plates were incubated for 42 h at 37°C in an atmosphere of 10% O₂, 5% CO₂, 85% N₂ and a humidity of 95%. Immediately after incubation, the plates were frozen and then thawed to lyse erythrocytes. The contents of each well were collected on standard filter microplates (Unifilter GF/B; Perkin Elmer, Meriden, NJ) and washed using a cell harvester (FilterMate Cell Harvester; Packard). Filter microplates were dried, and 25 µl of scintillation cocktail (Microscint O; Perkin Elmer) was placed in each well. Radioactivity incorporated by the parasites was measured using a scintillation counter (Top Count; Perkin Elmer).

The 50% inhibitory concentration (IC₅₀), i.e., the drug concentration corresponding to 50% of the uptake of [³H]hypoxanthine by the parasites in drug-free control wells, was determined by nonlinear regression analysis of log dose-response curves (Riasmart; Packard, Meriden, NJ). Data were analyzed after logarithmic transformation and expressed as the geometric mean IC₅₀, and 95% confidence intervals were calculated (Stata9; StataCorp LP, Tex.). Assessment of cross-resistance between the antimalarial drugs was estimated by a Spearman correlation coefficient (rho) and a coefficient of determination (*r*²).

The cutoff values, defined statistically (>2 standard deviations above the mean and/or after correlation with clinical failures) for in vitro resistance or reduced susceptibility to chloroquine, quinine, mefloquine, halofantrine, monodesethylamodiaquine, lumefantrine, dihydroartemisinin, atovaquone, cycloguanil, and pyrimethamine were 100 nM (18), 800 nM (4), 30 nM (16), 6 nM (3), 60 nM (5), 150 nM (32), 10.5 nM (33), 1,900 nM (24), 500 nM (6), and 2,000 nM (6), respectively.

RESULTS

One-hundred thirty-one samples with parasitemia ranging from 0.05 to 6.7% and examined within 72 h of collection were used to test drug susceptibility. For 14 isolates, there were not enough parasitized erythrocytes to screen all the antimalarial drugs. Twenty-one isolates with ratios of growth (maximum counts per minute/minimum counts per minute) of less than 4 were considered failures of in vitro culture. The following proportions of isolates were successfully cultured for each drug tested: 110 of 131 for chloroquine, quinine, mefloquine, atovaquone, and dihydroartemisinin; 109 of 131 for doxycycline; 104 of 131 for cycloguanil; 91 of 117 for lumefantrine; 90 of 117 for monodesethylamodiaquine; 40 of 53 for halofantrine; and 68 of 77 for pyrimethamine.

Average parameter estimates for the 11 compounds against the *P. falciparum* isolates are given in Table 1. The in vitro

TABLE 2. Selected correlation of in vitro responses of isolates of *P. falciparum* from Pointe-Noire ($r > 0.3$) to standard and new antimalarial drugs

Drug pair	No. of isolates tested	r	r^2	P
Halofantrine/mefloquine	40	0.882	0.777	<0.0001
Halofantrine/lumefantrine	32	0.749	0.560	<0.0001
Mefloquine/lumefantrine	91	0.654	0.428	<0.0001
Halofantrine/dihydroartemisinin	40	0.578	0.334	<0.0001
Mefloquine/dihydroartemisinin	110	0.543	0.294	<0.0001
Chloroquine/quinine	110	0.500	0.250	<0.001
Pyrimethamine/cycloguanil	67	0.473	0.224	<0.001
Halofantrine/quinine	40	0.352	0.124	0.0258
Lumefantrine/dihydroartemisinin	91	0.319	0.102	0.0020
Doxycycline/atovaquone	109	0.319	0.102	0.0007
Chloroquine/doxycycline	109	0.300	0.090	0.0016

resistance to chloroquine reached 75.5%. Twenty-eight percent of the isolates were intermediate or had reduced susceptibility to quinine. Seventy-six percent and 96% of the tested isolates showed in vitro resistance or intermediate susceptibilities to cycloguanil and pyrimethamine, respectively. Only 2% of the parasites demonstrated in vitro resistance to monodesethylamodiaquine.

Correlations of in vitro responses of isolates to standard and new antimalarial drugs with a coefficient of correlation greater than 0.3 are given in Table 2. Halofantrine, mefloquine, and lumefantrine demonstrated high correlation (coefficient of determination, r^2 , ranging from 0.428 to 0.777) (Fig. 1.). In vitro responses to lumefantrine and dihydroartemisinin showed an r^2 of 0.102. No cross-resistance was identified between responses to monodesethyl-amodiaquine and dihydroartemisinin ($r^2 = 0.001$) as well as atovaquone and cycloguanil ($r^2 = 0.001$).

DISCUSSION

This study reports the evaluation of the susceptibility of 11 standard and new antimalarial drugs against 110 isolates of *P. falciparum* from Pointe-Noire. Chloroquine was the most commonly used antimalarial drug in self treatment in rural or urban areas of the Republic of the Congo (27, 36, 37). One of the problems with parental administration of chloroquine is the lack of compliance. Increasing pressure to use chloroquine would probably induce chloroquine resistance. This investigation demonstrates a high prevalence of *P. falciparum* isolates resistant to chloroquine in vitro, a finding consistent with in vitro or in vivo data previously reported in Pointe-Noire (26, 27).

This chloroquine resistance is linked with a diminution of the susceptibility to quinine. Six percent of the isolates tested in our study have IC_{50} s higher than the defined cutoff of 800 nM. However, it should be noted that a lower cutoff for resistance to quinine (500 nM) has been used in several other studies (30). Using this cutoff, 28% of our isolates were resistant to quinine or had reduced susceptibility. One of the reasons for such decreased susceptibility could be an increasing use of quinine as presumptive treatment for uncomplicated

malaria in some health centers, often without respecting the recommended therapeutic protocol and dosage.

Only 2% of the isolates are resistant to the active metabolite of amodiaquine, monodesethylamodiaquine, confirming previous observations that amodiaquine might still be effective where chloroquine resistance is high (9, 23, 28). These data are consistent with previous in vitro observations in the Republic of the Congo (7). Nevertheless, this is in contrast with the results of in vivo studies carried in Pointe-Noire, with a 28% rate of clinical failure in 1987 (34), or in Brazzaville, with rates of clinical failure ranging from 29 to 43% in 1987 to 1996 (7, 10, 14). This raises the question of the relationship between the in vitro cutoff and the in vivo outcome. Similarly, studies in the Republic of the Congo and Gabon have shown no relationship between in vivo and in vitro susceptibility, as treatment failure was 30.7% and 40.5%, while only 4.6% and 5.4% of the isolates showed in vitro resistance to monodesethylamodiaquine (7, 1),

FIG. 1. Correlation of in vitro responses of isolates of *P. falciparum* from Pointe-Noire of halofantrine and mefloquine, halofantrine and lumefantrine, and mefloquine and lumefantrine.

indicating that the cutoff for amodiaquine resistance might be lower than the defined cutoff of 60 nM.

Few isolates, 7%, are resistant to mefloquine. These data are consistent with observations from Brazzaville from 1987 to 1993 (13, 15). There is no isolate showing in vitro resistance to halofantrine, lumefantrine, dihydroartemisinin, or atovaquone. The absence of resistance to halofantrine is in contrast with the data of Brasseur et al., which showed a high frequency and degree of resistance to halofantrine in the Republic of the Congo (8). However, these observations have never been shown again in the Republic of the Congo or in neighboring countries.

Our results report a high prevalence of isolates resistant to pyrimethamine and cycloguanil or with intermediate susceptibilities. Similar data (88%) were recently found in Pointe-Noire by the analysis of the mutations in dihydrofolate reductase (27). However, it seems that the combination of pyrimethamine plus sulfadoxine is still effective in the Republic of the Congo (26).

The correlation between halofantrine and mefloquine or lumefantrine and between mefloquine and lumefantrine might be partly explained by their similar chemical structure and consequently by their similar mode of action or mechanism of resistance (29).

The artemisinin derivatives are recommended to be associated with lumefantrine (31, 38), mefloquine (2), or amodiaquine (21, 35). In this study, we demonstrated in vitro cross-resistance between dihydroartemisinin and mefloquine or lumefantrine. The in vitro cross-resistance between lumefantrine and artemisinin derivatives has been observed previously (32). However, no in vitro resistance to both lumefantrine and dihydroartemisinin, monodesethylamodiaquine and dihydroartemisinin, or atovaquone and proguanil was demonstrated. It should be noted that there is no cross-resistance between dihydroartemisinin and monodesethylamodiaquine, which seems a good partner for artemisinin combination therapy.

In conclusion, since the level of chloroquine resistance in vitro linked to clinical failures exceeds an unacceptable upper limit and high rates of in vitro resistance to pyrimethamine and cycloguanil exist, diminution of the susceptibility to quinine and antimalarial drugs used in combination, such as amodiaquine, artemisinin derivatives, mefloquine, lumefantrine, or atovaquone, seem to be appropriate alternatives for the first-line treatment of acute, uncomplicated *P. falciparum* malaria. Further study is essential for useful decisions and changes in national drug policy.

ACKNOWLEDGMENTS

We thank the staff of the Centre Médical de Secours of Total Exploration et Production Congo in Pointe-Noire, the Département Médical International of Total in Paris, and Institut de Médecine Tropicale du Service de Santé des Armées in Marseilles for their technical support.

We acknowledge the financial support of the Délégation Générale pour l'Armement (grant 03CO001, no. 010808/03-6) and the Direction Centrale du Service de Santé des Armées. We thank Total for financial support for sample transport.

We have no conflicts of interest concerning the work reported in this paper. We do not own stocks or shares in a company that might be financially affected by the conclusions of this article. The conclusion of this article was not affected by finances.

REFERENCES

- Aubouy, A., J. Mayombo, A. Keundjian, M. Bakery, J. Le Bras, and P. Deloron. 2004. Lack of prediction of amodiaquine efficacy in treating *Plasmodium falciparum* malaria by in vitro tests. *Am. J. Trop. Med. Hyg.* 71: 294–296.
- Avila, J. C., R. Villaroel, W. Marquino, J. Zegarra, R. Mollinedo, and T. K. Ruebush. 2004. Efficacy of mefloquine and mefloquine-artesunate for the treatment of uncomplicated *Plasmodium falciparum* malaria in the Amazon region of Bolivia. *Trop. Med. Int. Health* 9:217–221.
- Basco, L. K., and J. Le Bras. 1992. In vitro activity of halofantrine and its relationship to other standard antimalarial drugs against African isolates and clones of *Plasmodium falciparum*. *Am. J. Trop. Med. Hyg.* 47:521–527.
- Basco, L. K., and J. Le Bras. 1994. In vitro susceptibility of Cambodian isolates of *Plasmodium falciparum* to halofantrine, pyronaridine and artemisinin derivatives. *Ann. Trop. Med. Parasitol.* 88:137–144.
- Basco, L. K., and J. Le Bras. 1993. In vitro activity of monodesethylamodiaquine and amopyroquine against African isolates and clones of *Plasmodium falciparum*. *Am. J. Trop. Med. Hyg.* 48:120–125.
- Basco, L. K., O. Ramilariisoa, and J. Le Bras. 1994. In vitro activity of pyrimethamine, cycloguanil, and other antimalarial drugs against African isolates and clones of *Plasmodium falciparum*. *Am. J. Trop. Med. Hyg.* 50:193–199.
- Brasseur, P., P. Agnemy, A. Same Ekobo, G. Samba, L. Favennec, and J. Kouamou. 1995. Sensitivity of *Plasmodium falciparum* to amodiaquine and chloroquine in central Africa: a comparative study in vivo and in vitro. *Trans. R. Soc. Trop. Med. Hyg.* 89:528–530.
- Brasseur, P., P. Bitsindou, R. S. Moyou, T. A. Eggelte, G. Samba, L. Penchenier, and P. Druilhe. 1993. Fast emergence of *Plasmodium falciparum* resistance to halofantrine. *Lancet* 341:901–902.
- Brasseur, P., R. Guigemde, S. Diallo, V. Guiyedi, M. Kombila, P. Ringwald, and P. Olliaro. 1999. Amodiaquine remains effective for treating uncomplicated malaria in west and central Africa. *Trans. R. Soc. Trop. Med. Hyg.* 93:645–650.
- Carme, B., A. Mbitsi, H. Moudzeo, M. Ndinga, and P. Eozenou. 1987. Drug-resistant falciparum malaria in Congo. 2. In vivo response to chloroquine and amodiaquine in schoolchildren in Brazzaville (November 1986). *Bull. Soc. Pathol. Exot.* 80:426–433.
- Carme, B., B. Yombi, J. C. Bouquety, H. Plassard, S. Nzingoula, J. Senga, and I. Akani. 1992. Child morbidity and mortality due to cerebral malaria in Brazzaville, Congo. A retrospective and prospective hospital-based study, 1983–1989. *Trop. Med. Parasitol.* 43:173–176.
- Carme, B., F. Gay, J. Chandenier, M. Ndounga, L. Ciceron, B. Ebikili, J. L. Smith, and M. Gentilini. 1991. Unexpected trend in chemosusceptibility of *Plasmodium falciparum* in Brazzaville, Congo. *Lancet* 338:582–583.
- Carme, B., F. Gay, M. Ndounga, M. P. Hayette-Gorremans, and J. C. Bouquety. 1995. In vitro sensitivity and clinical aspects of *Plasmodium falciparum* malaria in African children. *Trop. Med. Parasitol.* 46:270–274.
- Chambon, R., P. Lemardeley, C. Boudin, P. Ringwald, and J. Chandenier. 1997. In vivo sensitivity of *Plasmodium falciparum* to antimalarial drugs: results of a first test series by the OCEAC malaria control division. *Med. Trop.* 57:357–360.
- Chandenier, J., M. Ndounga, B. Carme, F. Gay, A. Mbitsi, M. P. Hayette, A. Stanghellini, J. Oko Ossoh, D. Baudon, and R. Caddy Zitsamélé. 1995. In vivo and in vitro chemosensitivity of *Plasmodium falciparum* in Brazzaville (Congo). *Cahier Santé* 5:25–29.
- Hatin, L., J. F. Trape, F. Legros, J. Bauchet, and J. Le Bras. 1992. Susceptibility of *Plasmodium falciparum* strains to mefloquine in an urban area in Senegal. *Bull. W. H. O.* 70:363–367.
- Lambros, C., and J. P. Vanderberg. 1979. Synchronization of *Plasmodium falciparum* erythrocytic stages in culture. *J. Parasitol.* 65:418–420.
- Le Bras, J., and P. Ringwald. 1990. *Plasmodium falciparum* chemoresistance. The situation in Africa in 1989. *Med. Trop.* 50:161–162.
- Le Bras, J., J. P. Coulaud, F. Bricaire, M. Le Bras, R. Roue, B. Grenier, and M. Fournon. 1985. Chloroquine-resistant falciparum malaria in Congo. *Lancet* ii:1071.
- Mabiala-Babela, J. R., C. Samba-Louaka, A. Mouko, and P. Senga. 2003. Morbidity in pediatric department (university hospital of Brazzaville): 12 years later (1989–2001). *Arch. Pédiatrie* 10:650–652.
- Martensson, A., J. Stroberg, C. Sisowath, M. I. Msellem, J. P. Gil, S. M. Montgomery, P. Olliaro, A. S. Ali, and A. Bjorkman. 2005. Efficacy of artesunate plus amodiaquine versus that of artemether-lumefantrine for the treatment of uncomplicated childhood *Plasmodium falciparum* malaria in Zanzibar, Tanzania. *Clin. Infect. Dis.* 41:1079–1086.
- Mayengue, P. I., M. Ndounga, M. M. Davy, N. Tandou, and F. Ntumi. 2005. In vivo chloroquine resistance and prevalence of the pfcr1 codon 76 mutation in *Plasmodium falciparum* isolates from the Republic of Congo. *Acta Trop.* 95:219–225.
- Menard, D., N. Madji, A. Manirakiza, D. Djalle, M. R. Koula, and A. Talarmin. 2005. Efficacy of chloroquine, amodiaquine, sulfadoxine-pyrimethamine, chloroquine-sulfadoxine-pyrimethamine combination, and

- amodiaquine-sulfadoxine-pyrimethamine combination in central African children with non-complicated malaria. *Am. J. Trop. Med. Hyg.* **72**:581–585.
24. Musset, L., B. Pradines, D. Parzy, R. Durand, P. Bigot, and J. Le Bras. 2006. Apparent absence of atovaquone/proguanil resistance in 477 *Plasmodium falciparum* from untreated French travellers. *J. Antimicrob. Chemother.* **57**:110–115.
 25. Nguyen-Dinh, P., I. K. Schwartz, J. D. Sexton, B. Egumb, B. Bolange, K. Rut, N. Nkutu-Pela, and M. Wéry. 1985. In vivo and in vitro susceptibility to chloroquine of *Plasmodium falciparum* in Kinshasa and Mbuji-Mayi, Zaire. *Bull. W. H. O.* **3**:325–330.
 26. Nsimba, B., D. A. Malonga, A. M. Mouata, F. Louya, J. Kiori, M. Malanda, D. Yocka, J. Oko-Ossho, S. Ebata-Mongo, and J. Le Bras. 2004. Efficacy of sulfadoxine/pyrimethamine in the treatment of uncomplicated *Plasmodium falciparum* malaria in Republic of Congo. *Am. J. Trop. Med. Hyg.* **70**:133–138.
 27. Nsimba, B., S. Jafari-Guemouri, D. A. Malonga, A. M. Mouata, J. Kiori, F. Louya, D. Yocka, M. Malanda, R. Durand, and J. Le Bras. 2005. Epidemiology of drug-resistance malaria in Republic of Congo: using molecular evidence for monitoring antimalarial drug resistance combined with assessment of antimalarial drug use. *Trop. Med. Int. Health.* **10**:1030–1037.
 28. Oduro, A. R., T. Anyorigi, A. Hodgson, P. Ansah, F. Anto, N. A. Ansah, F. Atuguba, G. Mumuni, and J. Amankwa. 2005. A randomized comparative study of chloroquine, amodiaquine and sulphadoxine-pyrimethamine for the treatment of uncomplicated malaria in Ghana. *Trop. Med. Int. Health* **10**:179–184.
 29. Peel, S. A., P. Bright, B. Yount, J. Handy, and R. S. Baric. 1994. A strong association between mefloquine and halofantrine resistance and amplification, overexpression, and mutation in the P-glycoprotein gene homolog (*pfmdr*) of *Plasmodium falciparum* in vitro. *Am. J. Trop. Med. Hyg.* **51**:648–658.
 30. Pettinelli, F., M. E. Pettinelli, P. Eldin de Pecoulas, J. Millet, D. Michel, P. Brasseur, and P. Druilhe. 2004. High prevalence of multidrug-resistant *Plasmodium falciparum* malaria in the French territory of Mayotte. *Am. J. Trop. Med. Hyg.* **70**:635–637.
 31. Piola, P., C. Fogg, F. Bajunirwe, S. Biraro, F. Grandesso, E. Ruzagira, J. Babigumira, I. Kigozi, J. Kiguli, J. Kyomuhenda, L. Ferradini, W. Taylor, F. Checchi, and J. P. Guthmann. 2005. Supervised versus unsupervised intake of six-dose artemether-lumefantrine for treatment of acute, uncomplicated *Plasmodium falciparum* in Mbarara, Uganda: a randomized trial. *Lancet* **365**:1467–1473.
 32. Pradines, B., A. Tall, T. Fusai, A. Spiegel, R. Hienne, C. Rogier, J. F. Trape, J. Le Bras, and D. Parzy. 1999. In vitro activities of benflumetol against 158 Senegalese isolates of *Plasmodium falciparum* in comparison with those of standard antimalarial drugs. *Antimicrob. Agents Chemother.* **43**:418–420.
 33. Pradines, B., C. Rogier, T. Fusai, A. Tall, J. F. Trape, and J. C. Doury. 1998. In vitro activity of artemether against African isolates (Senegal) of *Plasmodium falciparum* in comparison with standard antimalarial drugs. *Am. J. Trop. Med. Hyg.* **58**:354–357.
 34. Simon, F., J. Porte, F. Verdier, D. Guigon, C. Drouville, and J. Le Bras. 1987. Chemosensitivity of malaria in children from Pointe-Noire, Congo, first semester, 1986. *Bull. Soc. Pathol. Exot.* **80**:417–425.
 35. Sowunmi, A., F. A. Fehintola, A. A. Adediji, G. O. Gbotosho, E. Tambo, B. A. Fateye, T. C. Happi, and A. M. J. Oduola. 2005. Open randomized study of artesunate-amodiaquine vs. chloroquine-pyrimethamine-sulfadoxine for the treatment of uncomplicated *Plasmodium falciparum* malaria in Nigerian children. *Trop. Med. Int. Health* **10**:1161–1170.
 36. Talani, P., G. Samba, and G. Moyen. 2002. Management of child fever within malaria control in Brazzaville. *Bull. Soc. Pathol. Exot.* **95**:47–49.
 37. Talani, P., G. Samba, and G. Moyen. 2003. Management of children's fever at home in a rural area of Boko, Congo-Brazzaville. *Santé Publique* **15**:485–490.
 38. Van Vught, M., A. Brockman, B. Gemperli, C. Luxemburger, I. Gathmann, C. Royce, T. Slight, S. Looreesuwan, N. J. White, and F. Nosten. 1998. Randomized comparison of artemether-benflumetol and artesunate-mefloquine in treatment of multidrug-resistant falciparum malaria. *Antimicrob. Agents Chemother.* **42**:135–139.