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Therapeutic efficacy of sulfadoxine–pyrimethamine and the prevalence of molecular markers of resistance in under 5-year olds in Brazzaville, Congo

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Summary

OBJECTIVE To test the efficacy of sulfadoxine–pyremethamine (SP) monotherapy and establish the prevalence of mutations in *dhfr* and *dhps* in Brazzaville, Congo.
METHOD We recruited 97 patients aged 6–59 months with uncomplicated malaria who attended Tenrikyo public health centre. Eighty-three were followed until day 28. SP efficacy was determined by the WHO 28-day test and analysis of mutations in the *Plasmodium falciparum* dihydrofolate reductase (*pfdhfr*) and dihydropteroate synthase (*pfdhps*) genes.
RESULTS There were seven (8.4%) early treatment failures, 23 late treatment failures (27.7%), nine (10.8%) late parasitological failures and 44 (53%) adequate clinical and parasitological responses (ACPR). After polymerase chain reaction (PCR) analysis of 64 available samples, the corrected results there were 44 (68.8%) ACPR and 19 recrudescent cases (31.2%). Approximately, 97.5% of samples bore the Asn51Ile mutation, 66.2% the Cys59Arg mutation and 98.8% the Ser108Asn mutation. Mutations of *dhps* at positions 437 (Ala–Gly) and 436 (Ser–Ala) were found in 85% and 12.5% of samples. Quadruple mutations (*pfdhfr* triple mutations in codons 51, 59 and 108+ *pfdhps* mutation in 437) were found in 42 samples (52.5%) and associated with treatment failures.
CONCLUSION This high level of treatment failures and mutations in both genes calls for the urgent application of the new policy for malaria treatment to delay the spread of SP resistance.

keywords *Plasmodium falciparum*, sulfadoxine–pyrimethamine, efficacy, treatment failures, dihydrofolate reductase, dihydropteroate synthase, Brazzaville

Introduction

In forest and savannas areas of Congo, malaria transmission is perennial and intense. Congo Brazzaville officially adopted artemisinin-based combination therapies (ACTs) (WHO 2006) for malaria treatment in 2006. But chloroquine (CQ), amodiaquine (AQ), sulfadoxine–pyrimethamine (SP), quinine (Q) and monotherapies with artemisinin derivatives are still commonly used, as ACTs are not yet widely distributed and available in the health service. Surveillance of drug efficacy is essential for the National Malarial Control Programme to adapt to the changing epidemiology of drug-resistant malaria. As the emergence of CQ-resistant *Plasmodium falciparum*, the Congolese Ministry of Health has conducted regular surveys using the 1973 WHO protocol for asymptomatic children (WHO 1973; Carme *et al.* 1990, 1998; Chandenier *et al.* 1995) and *in vitro* isotopic micro-test (Brandicourt *et al.* 1991; Carme *et al.* 1991; Chandenier *et al.* 1995). High-level CQ resistance in asymptomatic carriers has been established without reference to the drug efficacy in symptomatic patients.

Since 2001, new WHO clinical protocols for the evaluation of therapeutic efficacy in symptomatic patients have been introduced in Congo. The first protocol with a 14-day follow-up period (WHO 1996) confirmed that in Brazzaville and Pointe-Noire, CQ is ineffective while SP remains highly effective (Nsimba *et al.* 2004). However, the 14-day *in vivo* test may overestimate the real efficacy of the drug because clinical and parasitological failures may

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appear after day 14. The second protocol (WHO 2003) evaluates therapeutic efficacy over a 28-day follow-up period.

In vitro models have shown that pyrimethamine resistance in *P. falciparum* is caused by a single Ser108Asn replacement in the dihydrofolate reductase (*dhfr*) gene and that the presence of triple or quadruple mutations (Asn51Ile, Cys59Arg, Ser108Asn and Ile164Leu) results in a high level of pyrimethamine resistance (Plowe *et al.* 1997; Wang *et al.* 1997; Curtis *et al.* 1998; Kublin *et al.* 1998). Commonly encountered mutations in the dihydropteroate synthase (*dhps*) gene associated with resistance to sulfadoxine include Ser436Ala, Ala437Gly and Lys540Glu (Triglia *et al.* 1997). Other amino acid substitutions may occur in *dhps* (Ser436Phe, Ala581Gly, Ala613Ser or Thr). Treatment failures with SP may be associated with 'quintuple' mutations, defined as the triple *dhfr* mutations Asn51Ile, Cys59Arg and Ser108Asn, and double *dhps* mutations (Ala437Gly and Lys540Glu) (Kublin *et al.* 2002; Alifrangis *et al.* 2003; Kyabayinze *et al.* 2003; Talisuna *et al.* 2004). We present the results of a study conducted in Brazzaville in 2003 using the 28-day WHO test to assess the efficacy of SP monotherapy and establish the prevalence of mutations in *dhfr* and *dhps*.

Materials and methods

This study took place in Brazzaville, which has a population of about 900 000 distributed between seven health districts. The study population consisted of about 300 000 residents of three southern health districts: Bacongo, Makélékélé and Mfilou. Bacongo and Makélékélé are meso-endemic, Mfilou is hyper-endemic (Trape 1987). Bacongo is urban, Makélékélé is a large semi-rural area, and Mfilou, on the periphery of Brazzaville, can also be considered semi-rural.

Patients were recruited at Tenrikyo public health centre in Makélékélé. Catchment patients live in the urban and semi-rural areas of the district, as well as in Bacongo and Massina (Mfilou). Febrile children aged from 6 to 59 months with presumptive malaria were referred to the laboratory for *Plasmodium* screening. Those with a parasite density between 2000 and 200 000 asexual parasites/μl were examined by a physician. Patients were recruited if (i) parental consent was obtained; (ii) the patient did not have danger signs (unable to drink or eat, vomiting more than twice in the last 24 h, recent history of convulsion, unconscious state or inability to sit or stand); (iii) absence of signs of severe malaria; (iv) a pack cell volume (PCV) >15%; (v) absence of other febrile illnesses and (vi) easy accessibility for possible home visits (WHO 2003). Before treatment, thick smears were prepared, and fingerprick capillary blood was collected on filter paper for molecular analysis and in a capillary tube for PCV determination.

Enrolled children were treated with the standard single dose (tablets) of Fansidar® (Hoffmann-La Roche, Basel, Switzerland): 25 mg/kg body weight of sulfadoxine and 1.25 mg/kg body weight of pyrimethamine. Treatment was given under the supervision of a member of our research group and observed for 30 min. If vomiting occurred during the observation period, treatment was repeated. Patients were excluded from the study in case of a second vomiting and referred to the health staff for alternative treatment. Patients received three doses of paracetamol (15 mg/kg) for home treatment and appointments for follow-up at the outpatient basis.

Patients were subsequently followed on days 1, 2, 3, 7, 14, 21 and 28. At visits between days 2 and 28, thick blood smears were prepared from fingerprick capillary blood to determine parasite density. Patients who were absent during the scheduled visits were seen at home. On and after day 7, fingerprick capillary blood was collected on filter paper for molecular analysis of the parasites from patients with parasitaemia.

Treatment outcomes were classified according to the WHO (2003) scheme for intense transmission area. Patients responding with early treatment failure (ETF), late clinical failure (LCF) and late parasitological failure (LPF) were treated with oral Q (8 mg base/kg body weight every 8 h for 5 days).

Blood samples collected on filter paper on day 0 and recrudescent malarial episodes were used to extract *P. falciparum* DNA for comparison by polymerase chain reaction (PCR). The size of the polymorphic central repeat region of merozoite surface protein 2 (MSP-2) and block 2 of MSP-1 of the pre- and post-treatment isolates were compared by agarose gel electrophoresis (Basco *et al.* 2004). Pre- and post-treatment parasites showing identical bands were considered as recrudescence, while different bands indicated new infection. Samples from patients responding with ETF (on or before day 3) were not analysed by PCR and were considered as recrudescent or persistent parasitaemia. Polymorphisms in *dhfr* codons 51, 59, 108 and 164, and *dhps* codons 436, 437, 540, 581 and 613 were determined by PCR and DNA sequencing (Basco *et al.* 2000; Tahar & Basco 2006).

Patients who withdrew from the study and those lost to follow-up during the 28-day follow-up period were excluded from analysis, and the proportions of ETF, LCF, LPF and adequate clinical and parasitological responses (ACPR) were calculated. The unadjusted treatment failure rate was defined as the number of patients responding with ETF, LCF or LPF divided by the total number of eligible

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patients. After excluding re-infections by PCR, the PCR-adjusted treatment failures were the total of ETF (assumed to be persistent parasitaemia), LCF and LPF because of recrudescence. The proportion of treatment failures (unadjusted by PCR) on day 14 was also calculated. Proportions and 95% confidence intervals (95% CI) were calculated using 6.04 Epi-info 6.04 fr (Centers for Disease Control and Prevention, Atlanta, GA, USA). The chi-squared test was used to compare proportions and determine the degree of association between gametocytes and treatment failures and between mutations and treatment failures. The level of significance was set at $P < 0.05$.

The Congolese Ministry of Health reviewed and approved our clinical studies. The WHO Secretariat Committee on Research Involving Human Subjects (SCRIHS) reviewed the study protocol and consent forms in French, English and local languages.

Results

A total of 97 patients (Table 1) were enrolled between October 2003 and February 2004 at the urban health centre of Tenrikyo located in the district of Makélékélé. Thirteen patients were excluded from the study: seven were withdrawn by the parents during the first 3 days because of hyperthermia, six received home treatment with either CQ or Q and one patient was referred to the district hospital. The following numbers of patients were lost to follow-up: one on day 2, one on day 3, one on day 7, one on day 14, one on day 21 and two on day 28. Some patients had persistent or recurrent fever on day 1 (19%) and/or day 2 (34%) (Table 2). These febrile episodes occurred despite the considerable decrease in parasitaemia. On day 3, 98% were afebrile. Parasitaemia decreased from the day 0 geometric mean of 30 900 asexual parasites/μl to 4100 asexual parasites/μl on day 2 and to 1220 asexual parasites/μl on day 3. Blood smears were positive in 66% on day 2 and in 48% on day 3. Side-effects reported by parents and/or observed during visits were mild and transient and indistinguishable from symptoms associated with malaria infection.

Table 1 Baseline characteristics of recruited patients

Recruited patients (*n*)	97
Mean age ± SD: months	30.8 ± 16.1
Range	6–67
>59-month old*: *n* (%)	4 (4.1)
Mean weight ± SD (kg)	11.7 ± 3
Range	6–20
Mean axillary temperature ± SD (°C)	38.5 ± 0.7
Range	37.5–40.0
Mean PCV: (%) ± SD	31.1 ± 3.7
Range	20–38
Geometric mean parasite density: parasites/μl	30 539
Range	2240–230 000
>200 000 parasites/μl: (*n*, %)	2 (2)
Antimalarial drug taken before inclusion	
Yes (%)	31 (32)
No (%)	64 (66)
Does not know (%)	2 (2)

PCV, pack cell volume.
*Range, 60–67 months.

Of seven cases responding with ETF, three presented with aggravation of clinical condition on day 2, one patient presented with fever and parasitaemia on day 3, and three afebrile patients presented with parasitaemia >25% of pre-treatment level on day 3 (Table 3). From days 4 to 14, nine additional failures (eight LCF and one LPF) were recorded. On day 28, there were 39 failures including 30 LCF and nine LPF. Among 32 late failures (LCF + LPF), 27 pre-treatment and post-treatment samples were available for genotyping. PCR analysis suggested that 13 patients presented recrudescence and 14 were re-infections. When patients with ETF are included, the PCR-corrected proportion of recrudescence was 31.2%. High gametocyte density and high proportion of gametocyte carriage were observed on days 7 and 14 (Table 2).

Of 76 patients followed until day 28 (except for seven ETF and 14 patients who were excluded or lost to follow-up), 12 (15.8%; five late failures and seven ACPR) were gametocyte-negative on day 0 and gametocyte-positive on day 28 and seven (9.2%; five late failures and two ACPR) were gametocyte-positive on days 0 and 28. Patients with gametocytaemia both at days 0 and 28 had no higher risk of late failure (relative risk, 0.58; 95% CI: 0.3–1.3; $P = 0.2$). Moreover, 42 patients (55.3%) did not develop gametocytes from days 0 to 28, whereas 15 patients (19.7%) with gametocytes on day 0 cleared gametocytaemia during the follow-up period. Among 19 patients with gametocytes at the end of follow-up, 10 (52.6%) responded with late failure. Twenty-one (36.8%) of 57 patients without gametocytaemia at the end of follow-up responded with late failure (relative risk, 1.4; 95% CI: 0.8–2.5%; $P = 0.8$).

Eighty pre-treatment isolates were analysed for *dhfr* (Table 4). A total of 97.5% had mutations of codon 51; 66.2% of codon 59 and 98.8% of codon 108. No mutation of codon 164 was found. 65% had double mutations of codons 51 + 59 and 97.5% of codons 51 + 108, while 65% had double mutations of codons 59 + 108 and triple mutations of codons 51 + 59. As for *dhps*, only the Ala437Gly mutation occurred frequently (85%), with the Ser436Ala mutation in 12.5% of isolates. Double mutations of codons 436 and 437 occurred in 6.2%. Lys540Glu

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Table 2 Evolution of axillary temperature and parasitaemia from days 0 to 7

	Day 0	Day 1	Day 2	Day 3	Day 7
No. patients	97	96	90	84	80
Mean temperature (°C) (range)	38.5 (37.5–40.0)	36.9 (36.0–39.8)	37.2 (36.0–40.2)	36.5 (36.0–38.9)	36.5 (36.0–38.5)
No. febrile patients (%)	97 (100)	18 (18.8)	31 (34.4)	2 (2.4)	2 (2.5)
Geometric mean parasite density (range)	30 900 (2240–230 000)		4100 (0–114 000)	1220 (0–31 400)	6 (0–300)
No. patients with positive smear (%)	97 (100)		59 (65.6)	40 (47.6)	2 (2.5)
Mean gametocyte density† (range)	62 (13–670)		64 (12–571)	57 (8–680)	181 (14–3040)
No. gametocyte carriers†: (%)	21/97 (22)		16/86 (19)	26/80 (33)	52/80 (65)*

Gametocytes density was calculated for gametocytes carrier only.
*$P < 0{,}01$, difference to days 1, 2 and 3.
†42/78 patients (54%) had 136 gametocytes/μL (range: 8–3085) on day 14; on day 21, 29/70 (41%) had 81 gametocytes/μl (range: 14–826); on day 28, 12/59 (20%) patients had 41 gametocytes/μl (range: 10–170).

Table 3 Clinical efficacy of SP in Brazzaville, Congo

	n	%	95% CI
Patients recruited	97		
Response on day 14			
Loss + withdrawn	11	11.3	5.8–19.4
Eligible patients	86	85.7	83.1–95.7
ETF	7	8.1	3.3–16.1
LCF	9	10.5	4.9–18.1
LPF	0	0	0
ACPR	70	81.4	71.6–89.0
Response on day 28: unadjusted with PCR			
Loss + withdrawn	14	14.4	8.1–23.0
Eligible patients	83	85.6	77.0–91.9
ETF	7	8.4	3.4–16.6
LCF	23	27.7	18.4–38.6
LPF	9	10.8	5.1–19.6
ACPR	44	53	41.7–64.1
Response on day 28 (PCR adjusted)			
Eligible patients	64	77.1	66.6–85.6
Failures	20	31.2	20.2–44.1
ETF	7/64	10.9	4.5–21.2
Recrudescence	13/64	20.3	11.3–32.2
ACPR	44/64	68.8	55.9–79.8

ETF, early treated failures; LTF, last treated failures; LPF, last parasitological failures; ACPR, adequate clinic and parasitological responses; PCR, polymerase chain reaction; CI, confidence interval.

substitution was not found. The 'quadruple' mutation [triple *dhfr* mutations (Asn51Ile, Cys59Arg, and Ser108-Asn) + Ala437Gly in *dhps*] was found in 52.5% of isolates.

Recrudescence was strongly associated with the presence of *dhfr* mutation Cys59Arg (Table 5). The prevalence of this mutation, either alone or in association with Ser108-Asn or with Asn51Ile, varied from 45.5% to 47.7% in parasites from patients responding with ACPR. A total of 88.2% of patients with recrudescence after SP therapy had Cys59Arg mutation alone or associated with other *dhfr* mutations ($P < 0.05$). The frequencies of Ser108Asn and Asn51Ile mutations, alone or in combination, were identical in patients responding with ACPR or recrudescence; 95.5–100% of isolates from patients responding with ACPR and those with recrudescence had a single mutation Ser108Asn or double mutations Ser108Asn + Asn51Ile. The prevalence of Ser436Ala mutation was not different in parasites isolated from these two patient groups on day 0: 11.4% (five of 44) from patients responding with ACPR and 11.8% (two of 17) from those with recrudescence. However, the prevalence of Ala437Gly was higher in parasites from patients with recrudescence (100%, 17/17) than in those from patients responding with ACPR (72.7%, 32/44) ($P < 0.05$). Among 44 patients with ACPR, double *dhps* mutations Ser436Ala and Ala437Gly were found in one isolate (2.3%), while these double mutations were found in two isolates (11.8%) among 17 patients with recrudescence. There were 15 patients (34.1%) with ACPR and 13 patients (76.5%) with recrudescence carrying the quadruple mutation, respectively ($P < 0.05$).

The quadruple mutation was present in seven of 18 gametocyte carriers responding with treatment failure because of recrudescence. Of these patients, two were infected with parasites carrying double *dhfr* mutations Asn51Ile and Ser108Asn and a single *dhps* mutation Ala437Gly. Among nine gametocyte carriers responding with ACPR, one patient was infected with parasites carrying the 'quadruple' mutation. Treatment failures were

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Table 4 Prevalence of *dhfr* and *dhps* mutations in pre-treatment *Plasmodium falciparum* isolates from Brazzaville, Congo

Genes	Mutations on codon	*n*/*N* (*N* = 80)	% (95% CI)
dhfr	51	78	97.5 (91.3–99.7)
	59	53	66.25 (54.8–76.4)
	108	79	98.8 (93.2–99.97)
	51 + 59	52	65 (53.5–75.3)
	51 + 108	78	97.5 (91.3–99.7)
	59 + 108	52	65 (53.5–75.3)
	51 + 59 + 108	52	65 (53.5–75.3)
dhps	436	10	12.5 (6.2–21.8)
	437	68	85 (75.3–92.0)
	436 + 437	5	6.25 (2.1–14.0)
dhfr + *dhps*			
3 *dhfr* + 1 *dhps*	108 + 51 + 59 + 437	42	52.5 (41.0–63.8)
3 *dhfr* + 2 *dhps*	108 + 51 + 59 + 436 + 437	5	6.25 (2.1–14.0)

n, number of isolates with mutations; *N*, number of isolates from pre-treated patients; 95% CI, 95% confidence interval: *dhfr*, dihydrofolate reductase; *dhps*, dihydropteorate synthase.

Table 5 Prevalence of mutations in *pfdhfr* in *pfdhps* and treatment outcomes in patients with uncomplicated malaria, Brazzaville

		ACPR (44 patients)		Recrudescence (17 patients)	
Genes	Mutations on codon	*n*	% (95% CI)	*n*	% (95% CI)
dhfr	51	42	95.5 (84.5–99.4)	17	100 (80.5–100.0)
	59	21	47.7 (32.5–63.3)	15	88.2 (63.6–98.5)
	108	43	97.7 (88.0–99.9)	17	100 (80.5–100.0)
	51 + 59	20	45.5 (30.4–61.2)	15	88.2 (63.6–98.5)
	51 + 108	42	95.5 (84.5–99.4)	17	100 (80.5–100.0)
	59 + 108	21	47.7 (32.5–63.3)	15	88.2 (63.6–98.5)
	51 + 59 + 108	20	45.5 (30.4–61.2)	15	88.2 (63.6–98.5)
dhps	436	5	11.4 (3.8–24.6)	2	11.8 (1.5–36.4)
	437	32	72.7 (57.2–85.1)	17	100 (80.5–100.0)
	436 + 437	1	2.3 (0.05–12.0)	2	11.8 (1.5–36.4)
dhfr + *dhps*	51 + 59 + 108 + 436	15	34.1 (20.5–49.9)	13	76.5 (50.1–93.2)
	51 + 59 + 108 + 436 + 437	1	2.3 (0.1–12.0)	2	11.8 (1.5–36.4)

ACPR, adequate clinic and parasitological responses; *dhfr*, dihydrofolate reductase; *dhps*, dihydropteorate synthase.

associated with quadruple mutation and gametocytaemia at the end of follow-up (relative risk, 4.4; 95% CI: 1.2–15.5%; $P < 0.05$).

Discussion

In 2006, Congo Brazzaville changed the national policy of antimalarial treatment and adopted ACT. Artesunate + AQ and artemether–lumefantrine are now recommended as the first- and second-line treatments of uncomplicated *falciparum* malaria respectively. However, while awaiting the procurement and distribution of these drug combinations throughout the country at an affordable price, monotherapies are still prescribed in the health system. In the circumstances, the spread of drug-resistant *P. falciparum* would appear to be inevitable. An operational system to monitor drug resistance in Congo is required urgently. Although SP is no longer officially recommended as the second-line drug in Congo, surveillance of the efficacy of this drug is important because it is one of the components of the ACT (artesunate + SP) available in most pharmacies or markets. Moreover, SP is expected to play a major role for the intermittent preventive treatment (IPT) of malaria in pregnant women and possibly in infants in Africa (White 2005).

The strategy of IPT using SP in pregnant women may become difficult to apply with the spread of antifolate-resistant *P. falciparum*. A recent study conducted in Kinshasa, Democratic Republic of Congo (located across the River Congo from Brazzaville) has shown that pregnant women under IPT remain infected with *P. falciparum* (Lukuka *et al.* 2006). Although SP efficacy in pregnant

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women under IPT cannot be extrapolated from data on SP efficacy in malaria-infected young children, the clinical response to SP was similar in these two population groups in Burkina Faso, with failure rates of 12.9% in young children *vs.* 13% in pregnant women (Coulibaly *et al.* 2006). Use of SP for IPT in antifolate-resistant zone may result in drug-induced gametocytogenesis (Sokhna *et al.* 2001; Tjitra *et al.* 2002; Sowunmi *et al.* 2005) and in higher transmission of gametocytes carrying the quadruple mutation (Mendez *et al.* 2002; Mockenhaupt *et al.* 2005). IPT should be based on an effective and relatively cheap drug with no fetotoxicity. At present, no such drug exists.

The best approach at present for carrying out surveillance of SP efficacy is to analyse blood samples collected from treated and subsequently followed patients for mutations. However, samples from febrile patients and asymptomatic carriers also provide valid data. This latter option has been applied in surveys conducted in Malawi (Bwijo *et al.* 2003), India (Biswas 2004), Democratic Republic of Congo (Cohuet *et al.* 2006) and Central African Republic (Menard *et al.* 2006), and is justified when previous surveys have provided evidence for SP resistance, rendering further prospective studies difficult because of ethical considerations. We combined an *in vivo* test and genetic marker analysis to assess the present status of SP efficacy. Since 2002, two surveys on SP efficacy have been conducted in the city of Brazzaville, one in 2001 (Nsimba *et al.* 2004) and ours in 2003. In 2001, another clinical study on SP in Kinshasa, Democratic Republic of Congo found a level of 94.6% of ACPR was reported (Kazadi *et al.* 2003). Sequence analysis of the isolates collected in Brazzaville in 2001 revealed a high prevalence of triple *dhfr* mutations and the absence of Lys540Glu and 'quintuple' mutations (Nsimba *et al.* 2005). This study found low efficacy of SP during the 28-day follow-up period, necessitating the withdrawal of the drug for second-line treatment of uncomplicated malaria in Congo. None of the Congolese isolates analysed in our study carried the *dhps* Lys540Glu mutation. In the absence of this mutation, low efficacy of SP has been associated with *dhfr* mutation Cys59Arg, *dhps* mutation Ala437Gly, and 'quadruple' mutations (Asn51Ile + Cys59Arg + Ser108Asn in *dhfr* and Ala437Gly in *dhps*).

We have established that SP resistance is a reality in Brazzaville. The situation is alarming and calls for further surveys in the Republic of Congo based on the analysis of isolates from patients with uncomplicated malaria. This approach is the most appropriate in a setting where monotherapies are still widely used and the spread of resistance is continuing because of drug pressure.

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Efficacité thérapeutique de la sulfadoxine–pyriméthamine et la prévalence des marqueurs moléculaires de résistance chez les moins de cinq ans à Brazzaville au Congo

OBJECTIF Evaluer l'efficacité de la sulfadoxine-pyriméthamine (SP) à Brazzaville, et determiner la pré valence des mutations dans les gènes codant pour la dihydrofolate rèductase (*pfdhfr*) et la dihydropteroate synthase (*pfdhps*) en 2003.

METHODES Sur 97 patients atteints de paludisme non compliqué à *Plasmodium falciparum* traités, avec une dose standard de SP, 83 ont été suivis jusqu'au jour 28. On a utilisé le test OMS de 28 jours et l'analyse des mutations dans les gènes codant pour la dihydrofolate réductase (*pfdhfr*) et la dihydropteroate synthase (*pfdhps*) de *Plasmodium falciparum*.

RÉSULTATS Sur les 83 patients suivis jusqu'au jour 28, 7 (8,4%) ont présenté un échec thérapeutique précoce, 23 (27,7%) un échec clinique tardif, 9 (10,8%) un échec parasitologique tardif et 44 (53%) une réponse clinique et parasitologique adéquate. Après l'analyse de 64 échantillons disponibles par la réaction de polymérisation en chaîne (PCR), les résultats corrigés ont donné 44 (68,8%) réponses cliniques et parasitologiques adéquates et 19 (31,2%) recrudescences. 97,5% d'échantillons portent la mutation Asn51Ile, 66,2% la mutation Cys59Arg et 98,8% la mutation Ser108Asn. Les mutations *dhps* aux positions 437 (Ala-Gly) et 436 (Ser-Ala) ont été trouvées dans 85% et 12,5% des échantillons respectivement. Les quadruples mutations (mutations triples dans *pfdhfr* aux positions 51, 59, et 108, plus la mutation dans *pfdhps* au niveau du codon 437) ont été trouvées dans 42 échantillons (52,5%) et étaient associées aux échecs de traitement.

CONCLUSION Ce taux élevé d'échecs au traitement et de mutations dans les deux gènes appelle à l'application urgente de la nouvelle politique de traitement du paludisme afin de retarder la propagation de la résistance à la SP.

mots clés *Plasmodium falciparum,* Sulfadoxine–pyriméthamine, efficacité, échecs de traitement, dihydrofolate réductase, dihydropteroate synthase, Brazzaville

Eficacia terapéutica de la sulfadoxina-pirimetamina y la prevalencia de marcadores moleculares de resistencia en niños menores de cinco años en Brazzaville, Congo

Hemos realizado un estudio de la eficacia de la sulfadoxina-pirimetamina (SP) en Brazzaville durante el 2003, utilizando la prueba de 28-días de la OMS y el análisis de mutaciones en los genes de la dihidrofolato reductasa de *Plasmodium falciparum* (*dhfrpf*) y la dihidropteroato sintetasa (*dhpspf*). Los niños menores de 5 años con malaria no complicada por falciparum fueron tratados con la dosis estándar de SP. De los 97 pacientes reclutados, a 83 se les siguió hasta el día 28. Hubo 7 (8.4%) fallos de tratamiento tempranos, 23 fallos de tratamiento tardíos (27.7%), 9 (10.8%) fallos parasitológicos tardíos y 44 (53%) respuestas clínicas y parasitológicas adecuadas (RCPA). Después del análisis mediante PCR de las 64 muestras disponibles, los resultados corregidos fueron los siguientes: 44 (68.8%) RCPA y 19 (31.2%) casos de recrudescencia. La prevalencia de mutaciones en *dhfr* fue muy alta, con un 97.5% de las muestras presentando la mutación Asn51Ile, 66.2% la mutación Cys59Arg, y 98.8% la mutación Ser108Asn. Las mutaciones de la *dhps* en las posiciones 437 (Ala a Gly) y 436 (Ser a Ala) se encontraron en un 85% y 12.5% de las muestras, respectivamente. Las mutaciones cuádruples (mutaciones triples *dhfrpf* en los codones 51, 59, y 108 + mutación *dhpspf* en 437) fueron halladas en 42 muestras (52.5%) y estaban asociadas con los casos de fallo terapéuticos. Este alto nivel de fallo terapéutico y las mutaciones en ambos genes hacen que sea urgente la aplicación de una nueva política para el tratamiento de la malaria, con el fin de retrasar la dispersión de la resistencia a SP.

palabras clave *Plasmodium falciparum*, sulfadoxina-pirimetamina, eficacia, fallo terapéutico, dihidrofolato reductasa, dihidropteroato sintetasa, Brazzaville

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