Susceptibility of *Plasmodium falciparum* to the drugs used to treat severe malaria (quinine) and to prevent malaria (mefloquine, cycloguanil) in Comoros Union and Madagascar

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Objectives. To monitor the sensitivity of *Plasmodium falciparum* to the drugs used to treat severe malaria and to prevent malaria in Comoros and Madagascar.

Design. We used the *in vitro* isotopic method to test the sensitivity of *P. falciparum* to quinine, mefloquine and cycloguanil.

Results. We tested fresh isolates of *P. falciparum*, collected from patients living in urban, suburban and rural areas and suffering from uncomplicated malaria in 2001, against at least one of the antimalarials cited above. In both countries all of the successfully tested isolates were sensitive to quinine ($N = 243$) and to cycloguanil ($N = 67$). The mean IC50 ranged from 85.7 to 133.7 nM for quinine. For cycloguanil, the mean IC50 ranged from 1.4 to 20.2 nM and the highest IC50 value (102.5 nM) was recorded in Comoros. Only 0.9% (1/110) of the informative isolates from Madagascar were mefloquine-resistant (0/18 in Comoros). The mefloquine mean IC50s were 8.2 nM, 14.1 nM and 11.6 nM respectively in the rural, suburban and urban areas of Madagascar, and 5.9 nM in Comoros. A positive correlation was found between quinine and mefloquine IC50s ($N = 127$, $r = 0.48$, $p < 10^{-6}$), but *in vitro* mefloquine was 6 - 16 times more potent than quinine. No correlation was noticed between the activities of quinine and cycloguanil or between the activities of mefloquine and cycloguanil.

Conclusion. We therefore advocate the use of a full-course regimen of quinine, as recommended by the World Health Organisation (WHO), to treat above all severe malaria in Madagascar and Comoros. Our results also demonstrate that the use of mefloquine- and cycloguanil-based antimalarials is still justified to prevent malaria in both countries, mainly in the case of travellers.

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Knowledge of the efficacy of antimalarial drugs in malarial countries is vital in adapting malaria chemotherapy and chemoprophylaxis strategies. Chloroquine resistance now occurs almost in every area where *Plasmodium falciparum* circulates. Resistance to most of the commonly used antimalarials including mefloquine has been documented.[1] Over the past five decades, chloroquine has been used to cure and/or to prevent malaria in Comoros Union and in Madagascar. Chloroquine resistance is not currently a real concern in Madagascar, even though cases of chloroquine treatment failure and chloroquine chemoprophylaxis failure led to the over-hasty description of resistance.[2-4] In contrast, in the Comoros Union the prevalence of chloroquine resistance is high.[5] Chloroquine treatment fails in 60% of children under 5 years of age in the three islands of the archipelago (Silai *et al.* — unpublished data).

Quinine is the main treatment for severe malaria in Comoros and Madagascar. Mefloquine and cycloguanil-based antimalarial drugs are recommended for the prevention of malaria, particularly for travellers.[6,7] Very few *in vivo* or *in vitro* tests have been done to assess the chemosusceptibility of *P. falciparum* in this part of the Indian Ocean subregion. In 2000, the Madagascan Ministry of Health renewed the challenge to get updated data on the effectiveness of antimalarial drugs, in collaboration with the Malaria Research Group of the Institut Pasteur de Madagascar (IPM). As part of this project, *in vitro* tests were carried out on fresh *P. falciparum* isolates to monitor their susceptibility to the drugs recommended and used for severe malaria therapy and for malaria prevention in Madagascar. In early 2001, the Comorian health policy makers asked the IPM for expert help in assessing malaria resistance. We report here on the sensitivity of *P. falciparum* in both countries to quinine, mefloquine and cycloguanil.

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Materials and methods

Study sites and collection of *P. falciparum* isolates

Madagascar is a large island measuring 587 000 km^2. *P. falciparum* isolates were collected from a number of sites in the rural, suburban and urban areas within the three regions of different *Plasmodium* transmission patterns.[8] The collection sites were, from north to south, Antsiranana, Mahajanga, Ranohira, Morondava and Toliara in the west region (malaria transmission period < 6 months per year); Antananarivo and Saharevo in the central region (malaria transmission period < 4 months per year and unstable); and Sambava, Sainte Marie, Toamasina, Mananjary and Esana in the east region (malaria transmission period > 6 months per year). The town of Moroni was the collection site in the Comoros Union. Thick blood smears were prepared for each outpatient attending the health centres at study sites with suspected malaria. These smears were Giemsa-stained and examined with a light microscope to determine whether malaria parasites were present. Patients presenting with signs and symptoms of severe and complicated malaria, as defined by the World Health Organisation (WHO),[9] were excluded. Blood samples (2 - 5 ml) were collected from consenting patients (or patients whose guardians or parents gave consent) by venepuncture. The samples were collected in citric acid dextrose. They were transported at +4°C to the malaria research group at the IPM in Antananarivo within 48 hours of the blood collection. Patients were treated for malaria by the local physician according to the recommendation of the national policy, without immediate or delayed follow-up. The Madagascan and the Comorian Ministries of Health approved the study protocol.

Antimalarial drugs tested

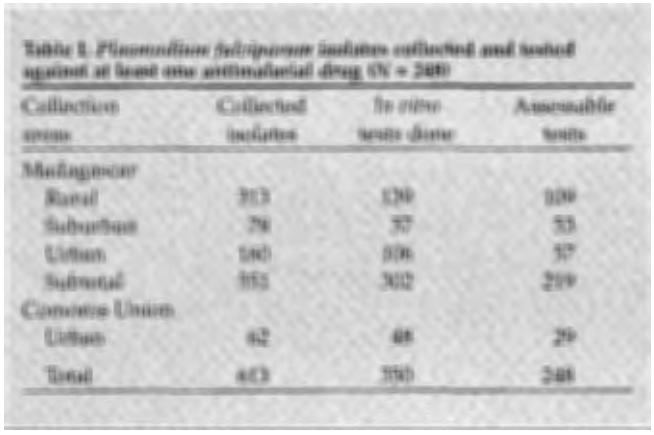
Cycloguanil was obtained from Zeneca, quinine base from Sigma Chemicals and mefloquine from Roche Products. Sterile stock solutions were prepared in methanol/water and serial dilutions were made in distilled water. Test concentrations ranged from 0.3 to 32 000 nM for cycloguanil, from 2.5 to 400 nM for mefloquine and from 25 to 3 200 nM for quinine. *In vitro* chemosensitivity tests were performed according to the isotopic microtest method[10] as described elsewhere.[11] Rosewell Park Memorial Institute (RPMI) 1640 medium (Gibco, BRL, France) was used to test the quinoline-containing antimalarials (mefloquine and quinine). RPMI medium without *p*-aminobenzoic and without folic acid (Biowhittaker Europe, A Cambrex Company, Belgium) was used to test cycloguanil. Both media were supplemented with 10% (volume/volume) non-immune human-type AB-positive serum. [^{3}H]-hypoxanthine was added to a concentration of 1 µCi per well on the culture plate. Characterised *P. falciparum* clones 3D7 and FCM29, maintained in continuous culture in our laboratory, were used as references to test the quality of each batch of dosed plates.

Data analysis

The concentration of the test antimalarials required to inhibit the growth of 50% of the parasites (IC50) was calculated using regression analysis of log concentration/response probit curves. An isolate was considered to be resistant when the IC50 was > 800 nM for quinine, > 50 nM for mefloquine, and 500 nM for cycloguanil.[11-13] Results are expressed as the mean IC50 and 95% confidence intervals (95% CI). Epi-Info 6 software was used to compare mean IC50s. The correlation between the *in vitro* sensitivity of *P. falciparum* to two different drugs, based on IC50 values, was analysed using Pearson's correlation.

Results

Between January and December 2001, 613 blood samples containing *P. falciparum* were sent to the Malaria Research Group of the IPM. Only monospecific *P. falciparum* isolates were tested when at least 2 000 ring stage parasites were detected per microlitre of blood, and if patients had not recently (< 7 days) taken antimalarial drugs. Thus, 350 isolates (57.1%) were tested against at least one of the antimalarials cited above. The results from areas of the same category (rural, suburban and urban) were considered together (Tables I and II).



Quinine

In Madagascar, successfully tested *P. falciparum* isolates were sensitive to quinine (N = 215). However, the differences between the mean IC50s were statistically significant ($p < 10^{-6}$) in the rural, suburban and urban areas of Madagascar. The mean IC50 was 133.7 nM (95% CI: 107.5 - 160 nM) in the urban area of Madagascar, which is 1.56 times higher than the mean IC50s observed in the rural area. The highest IC50 (548.2 nM) was detected in Madagascar. In Comoros, the 28 successfully tested isolates were sensitive to quinine (mean IC50 = 96.2 nM, 95% CI: 68.7 - 123.7 nM).

Table II. Mean IC50s with 95% confidence intervals (95% CI) for quinine, mefloquine and cycloguanil

	Study areas			
	Madagascar			Comores
Antimalarial drugs tested	Rural	Suburban	Urban	(urban)
Quinine				
Assessable tests	107	53	55	28
Mean IC50s (nM)	85.7	90.2	133.77	96.2
IC50s 95% CI	75.6 - 95.9	76.3 - 104	107.5 - 160	68.7 - 123.7
Highest IC50 (nM)	280.4	214.3	584.2	313.2
Resistant isolates (*N*)	0	0	0	0
Mefloquine				
Assessable tests	56	22	32	18
Mean IC50s (nM)	8.2	14.1	11.6	5.9
IC50s 95% CI	6.8 - 9.6	8.7 - 19.5	9.3 - 13.9	3.4 - 8.4
Highest IC50 (nM)	27.8	53.2	29.8	26.3
Resistant isolates (*N*)	0	1	0	0
Cycloguanil				
Assessable tests	23	14	19	11
Mean IC50s (nM)	6.1	1.4	7.2	20.2
IC50s 95% CI	1.7 - 10.6	0.9 - 1.9	3.1 - 11.3	1.6 - 38.8
Highest IC50 (nM)	48.2	3.2	35.1	102.5
Resistant isolates (*N*)	0	0	0	0

Mefloquine

Successfully tested *P. falciparum* isolates were mefloquine-sensitive, except 1 of the 110 (0.9%) from Madagascar. This parasite of mefloquine-resistant phenotype was collected from the suburban area of Mananjary, in the south-eastern part of the island. The mean IC50s were 8.2 nM (95% CI: 6.8 - 9.6 nM), 14.1 nM (95% CI: 8.7 - 19.5 nM) and 11.6 nM (95% CI: 9.3 - 13.9 nM) respectively in the rural, suburban and urban areas. The differences between the mean IC50s were statistically significant between the three areas categories in Madagascar ($p = 0.007$). Nevertheless, virtually all *P. falciparum* isolates from this part of the Indian Ocean region were sensitive to mefloquine. For all regions combined, 82.8% (106/128) of the IC50 values were below 15 nM (94.4% in Comoros and 80.9% in Madagascar). The only IC50 value over 15 nM (1/18) recorded in Comoros was 26.3 nM.

Cycloguanil

Of the 139 tests carried out with cycloguanil, 67 (48.2%) were assessable. The 56 successfully tested Madagascan isolates were highly sensitive to cycloguanil. The IC50s were always below 50 nM. However, 1 of the 11 Comorian isolates showed decreased sensitivity to cycloguanil, with an IC50 value > 100 nM. The differences between the mean IC50s in Madagascar and in Comoros were not significant.

Correlation between drug activities

Table III shows the correlations between drug activities based on the IC50 values. Data from Madagascar and Comoros were considered together. Significant positive correlation was found between the susceptibility of *P. falciparum* to mefloquine and to quinine in this part of the Indian Ocean subregion ($N = 127$, Pearson's $r = 0.48$, $p < 10^{-6}$). The mean quinine IC50/mean

Table III. Correlations between *Plasmodium falciparum* isolate sensitivities to quinine, mefloquine and cycloguanil *in vitro* in Comoros Union and Madagascar

Drug pair	Isolate number	*r*	r^2	*p*-level*
Quinine-mefloquine	127	0.48	0.234	$< 10^{-6}$
Quinine-cycloguanil	63	- 0.09	0.009	0.47
Mefloquine-cycloguanil	62	- 0.16	0.026	0.21

* Correlation is significant for $p < 0.05$.

mefloquine IC50 ratio ranged from 6.4 to16.3. No sign of correlation was observed between the activities of quinine and cycloguanil or between the activities of mefloquine and cycloguanil.

Discussion

We used the *in vitro* sensitivity test to assess the intrinsic activity of antimalarial drugs. *In vitro* and *in vivo* investigations recently confirmed that chloroquine resistance of *P. falciparum* is a concern in Comoros,[5] whereas *P. falciparum* chloroquine susceptibility and chloroquine therapeutic efficacy are still satisfactory in Madagascar.[4,14,15] Interestingly, Jambou *et al.*[16] reported the therapeutic effectiveness of a 3-day regimen of oral quinine to treat uncomplicated malaria in Madagascar. The efficacy of quinine in areas of drug resistance in Africa[17-20] supports the use of quinine for the management of recrudescent *P. falciparum* following the use of chloroquine or other antimalarial drugs.

Our results show that Comorian and Madagascan *P. falciparum* strains are sensitive to quinine. These results suggest that quinine can be used to treat either severe or recrudescent *P. falciparum* malaria in these countries — using the full-course quinine regimen as recommended by the WHO.[21]

However, the unsupervised use of quinine has been observed in urban areas of Madagascar. It is common for suspected and confirmed cases of malaria to be treated with quinine (three daily intramuscular injections) in main towns. Most of the time the dose administered is insufficient. We found that the quinine IC50s were almost 1.6 times higher in urban areas than rural areas. This raises the question as to whether drug pressure related to quinine use in urban areas may select parasites with decreased sensitivity to quinine. It is therefore essential that the therapeutic efficacy of quinine also be monitored. The annual publication of national standard practice guidelines for the use of antimalarials may help to control the misuse of quinine and keep health carers, physicians and practitioners informed.

Our results also show that mefloquine was 6.4 - 16.3 times more potent than quinine and that there is a correlation between the sensitivity of *P. falciparum* to the two drugs. The structural similarities between quinine and mefloquine might explain this correlation. All successfully tested Comorian *P. falciparum* isolates were sensitive to mefloquine and only 1 (0.9%) mefloquine-resistant *P. falciparum* isolate was detected in Madagascar. Even if the IC50 threshold for mefloquine resistance is still unclear, our previous studies[15,22] also detected a low rate (3.9% in 1997 and 2% in 1999) of mefloquine-resistant *P. falciparum* in Madagascar. Mefloquine is mainly advocated for the prevention of malaria in travellers in the malaria areas of the Indian Ocean region. The occurrence of potentially mefloquine-resistant parasites will hamper the prevention strategy. The need for relevant and up-to-date data on the effectiveness of mefloquine means that we need to monitor its efficacy for prophylaxis in pilot studies. *In vitro* sensitivity testing should be continued until the mechanism underlying mefloquine resistance is understood.

Regarding the readout of the *in vitro* sensitivity/resistance of isolates to cycloguanil, low tritium-labelled hypoxanthine incorporation was noted in non-assessable tests (less than 1 000 counts/min in the controls). This might be due to the lack of folic and *p*-aminobenzoic acid in culture medium used to test antifolates.[12] Even though the number of assessable tests was limited, our results showed that *P. falciparum* is highly sensitive to this drug in Madagascar. Few isolates with decreased sensitivity to cycloguanil seem to occur in Comoros. Basically, the combination of chloroquine plus cycloguanil for prevention of malaria is still recommended in Madagascar. In Comoros, we presume that the same combination can currently be used and that mefloquine is still recommended to prevent malaria. The increasing prevalence of chloroquine-resistant *P. falciparum* in the Comoros commonly leads to increased use of the combination sulfadoxine-pyrimethamine for malaria treatment. As cycloguanil is also a dihydrofolate reductase (DHFR) inhibitor, like pyrimethamine, particular attention should be paid to the use of cycloguanil-based antimalarials in the Comoros over the next decade. Molecular approaches[14,23] should be used for surveillance of *P. falciparum* cycloguanil resistance/susceptibility.

Conclusion

Drug resistance in malaria is a vital public health concern. The resistance pattern in each country provides useful guidance concerning treatment and prevention, because no bedside methods are available to assess antimalarial drug susceptibility. Our *in vitro* study demonstrated that *P. falciparum* parasites are generally sensitive to quinine, mefloquine and cycloguanil in Comoros Union and in Madagascar. These results justify the use of quinine for the treatment of severe malaria, and the prescription of mefloquine- and cycloguanil-based antimalarials for malaria prophylaxis in this part of the Indian Ocean.

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Oral trimethoprim-sulfamethoxazole in the treatment of cerebral toxoplasmosis in AIDS patients — a prospective study

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Toxoplasma encephalitis is the commonest cause of intracranial mass lesions in AIDS patients. Effective therapy includes pyrimethamine plus sulfadiazine, clindamycin with pyrimethamine, and co-trimoxazole. This study examines the efficacy of oral co-trimoxazole in 20 AIDS patients with toxoplasmosis and seeks to confirm the experience of Torre *et al*.

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Toxoplasmosis is the most frequent cause of focal cerebral lesions in patients with AIDS.[1] Current therapy for acute cerebral toxoplasmosis is a combination of pyrimethamine plus sulfadiazine.[2] Alternative agents include clindamycin, clarithromycin and azithromycin.[3,4] In addition to the significant adverse effect profile of the pryimethamine combination, necessitating folinic acid replacement, none of these agents is available in intravenous form in most public service hospitals within the province of KwaZulu-Natal. The combination of trimethoprim-sulfamethoxazole (co-trimoxazole) has been shown to possess antitoxoplasmic activity *in vitro*[3] and *in vivo*.[5,6] The latter study,[6] a retrospective appraisal of intravenous therapy, showed a favourable outcome in 87% of treated patients. The aim of the present study was to prospectively evaluate the efficacy of oral co-trimoxazole in acute cerebral toxoplasmosis.