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IN VITRO SUSCEPTIBILITY OF *PLASMODIUM FALCIPARUM* ISOLATES FROM MYANMAR TO ANTIMALARIAL DRUGS

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Abstract. *In vitro* drug susceptibility profiles were assessed in 75 *Plasmodium falciparum* isolates from 4 sites in Myanmar. Except at Mawlamyine, the site closest to the Thai border, prevalence and degree of resistance to mefloquine were lower among the Myanmar isolates as compared with those from Thailand. Geometric mean concentration that inhibits 50% (IC_{50}) and 90% (IC_{90}) of Mawlamyine isolates were 51 nM (95% confidence interval [CI], 40–65) and 124 nM (95% CI, 104–149), respectively. At the nearest Thai site, Maesod, known for high-level multidrug resistance, the corresponding values for mefloquine IC_{50} and IC_{90} were 92 nM (95% CI, 71–121) and 172 nM (95% CI, 140–211). Mefloquine susceptibility of *P. falciparum* in Myanmar, except for Mawlamyine, was consistent with clinical-parasitological efficacy in semi-immune people. High sensitivity to artemisinin compounds was observed in this geographical region. The data suggest that highly mefloquine-resistant *P. falciparum* is concentrated in a part of the Thai-Myanmar border region.

INTRODUCTION

In Thailand, malaria is only prevalent along some areas of the country's international borders. However, the disease remains a leading public health threat in Myanmar (formerly Burma). The problem of multidrug-resistant malaria on the Thai-Myanmar border is well recognized.[1,2] Chloroquine resistance is believed to have spread westward from the Thai-Cambodian border across Thailand to Myanmar and other parts of Asia and Africa beginning ~ 4 decades ago.[3] In the late 1980s and early 1990s, migration of gem miners from the Thai-Cambodian border to the Thai-Myanmar border resulted in a rapid loss of mefloquine efficacy in both borders of Thailand.[2,4] Concerns have been raised about the spread of mefloquine resistance westward from the Thai border after the example of chloroquine resistance spread.

However, data on the status of antimalarial drug resistance in Myanmar are scarce. It is also not known if reduced sensitivity to artemisinin derivatives has developed, and if so, whether it indeed originated in Myanmar.[5] Such a possibility is feasible because artemisinin compounds have been available without control in Myanmar for more than a decade. In the current study, we compared *in vitro* susceptibility assay data from *Plasmodium falciparum* isolates collected from 4 geographically diverse areas in Myanmar to those obtained from Thailand and Bangladesh near their borders with Myanmar. Few reports on the *in vitro* susceptibility status of Myanmar isolates have been published in the past 2 decades.[6,7]

SUBJECTS AND METHODS

Collection of isolates. We undertook malaria surveys in Lashio (Shan State) and Mawlamyine (Mon State) in November 1997 and in Myitkyina (Kachin State) and Dawei (Tanintharyi Division) in September 1998 (Figure 1). Malaria diagnosis was made by microscopy with an interference filter system for the examination of acridine orange-stained thin blood smears.[8] We screened both symptomatic and asymptomatic volunteers. After we obtained verbal consent, we collected 2 mL blood under sterile technique by venipuncture with Vacutainers with heparin (Becton Dickinson, Franklin Lakes, NJ) from volunteers aged ≥ 10 years (with parental consent among those aged < 20 years) found to be positive for *P. falciparum.* Volunteers then received antimalarial drugs according to standard local regimens from Myanmar health authorities. Exclusion criteria included known bleeding tendency or history of antimalarial drug use within the past 2 weeks. The study was approved by Nagoya University School of Medicine ethical review committee and the Myanmar Ministry of Health.

We collected a total of 116 isolates (Table 1). The isolates were cryopreserved in a dry liquid nitrogen shipper and transported to Bangkok, where they were thawed 1–3 months later.

In Thailand, isolates were also collected as a part of our continuing surveillance[9] from malaria clinics at 3 towns along the Thai-Myanmar border, namely Maesod (the upper central part), Sangkhlaburi (the lower central part), and Ranong (the southern part), as well as from a malaria clinic in Yala, a southern province bordering Malaysia. Maesod is known for its high prevalence of multidrug-resistant malaria and exceptionally high level of mefloquine resistance.[2,9] Additionally, we obtained isolates from falciparum malaria patients detected by microscopy of Giemsa-stained slides at health clinics near Chittagong, Bangladesh. These isolates were similarly cryopreserved and transported to Bangkok.

Cryopreservation. In the field, blood was centrifuged, plasma and buffy coat removed, and red blood cells (RBCs) washed twice in RPMI 1640 (with $NaHCO_3$). The RBCs were resuspended in equal volume of freezing solution (D-sorbitol–glycerol) and the suspension divided into 1-mL aliquots in vials for cryopreservation.

***In vitro* adaptation.** At the Armed Forces Research Institute of Medical Sciences (AFRIMS), the aliquots were removed from storage and placed in a 37°C water bath. The thawed RBC suspension was quickly added to 5 volumes of prewarmed (37°C) sterile 3.5% NaCl solution, mixed well, and centrifuged (relative centrifugal force = 405 × *g*, 5

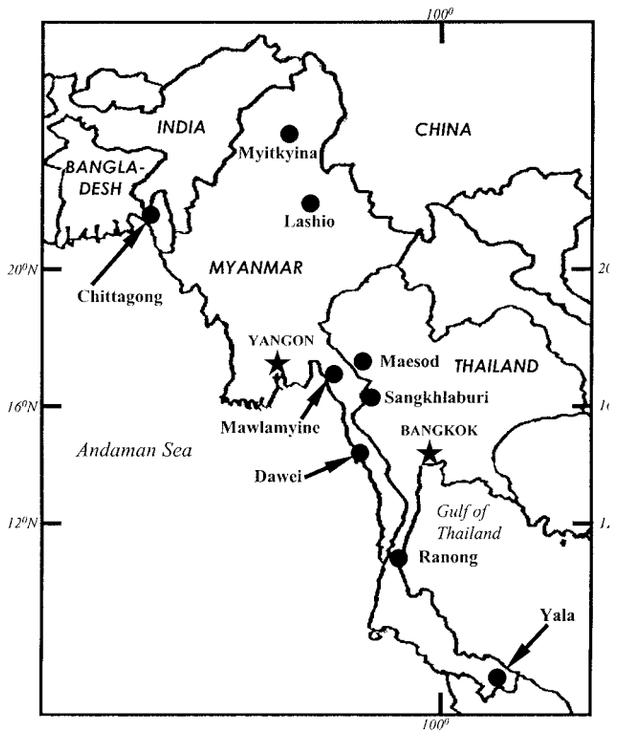

FIGURE 1. Map of Myanmar showing the 4 *Plasmodium falciparum* isolate collection sites and the Thai and Bangladeshi towns near the Myanmar borders, where isolates were also collected for *in vitro* susceptibility comparisons.

min). The supernatant was decanted, and packed RBCs were washed twice with RPMI 1640 (with $NaHCO_3$). The washed RBCs were resuspended in complete medium (RPMI 1640 + $NaHCO_3$, supplemented with 10% heat-inactivated serum) and placed in a 25-cm^3 tissue culture flask in a total volume of 5 mL of 5% RBC suspension. The flask was flushed with a gas mixture of 5% CO_2, 5% O_2, and 90% N_2 and placed in an incubator (37°C). The culture medium was changed once a day, and Group O RBCs were added to maintain the 5% cell suspension. Parasite growth was monitored by Giemsa-stained thin smear examination.

***In vitro* microtests.** Once an optimum density of 1% parasitized RBCs was reached, usually within 10–20 days, the cultured parasites were subjected to *in vitro* microtests by a radioisotope technique slightly modified from those previously reported.[10,11] In this semiautomated microdilution technique, we used 1.5% hematocrit and 0.5% initial parasitemia. Each drug was serially diluted 2-fold in a standard microtitration plate for a total of 7 concentrations. The drugs and their respective final concentration ranges in cell-medium mixture were as follows: 1) chloroquine diphosphate 7.144–457.200 ng/mL, corresponding to 13.848–886.275 nM; 2) quinine sulfate dihydrate 21.438–1,372.000 ng/mL, corresponding to 54.615–3,495.274 nM; 3) mefloquine hydrochloride 1.948–124.700 ng/mL, corresponding to 4.696–300.643 nM; 4) artesunate 0.265–16.930 ng/mL, corresponding to 0.689–44.040 nM; and 5) artemisinin 0.265–16.930 ng/mL, corresponding to 0.939–59.964 nM.

Suspensions of parasites and drugs were incubated in microtitration plates at 37°C for 24 hr before the plates were pulsed with [^{3}H]-hypoxanthine and reincubated for additional 18 hr. Each microtitration plate was then harvested, and scintillation counts were determined with a Filter Mate Harvester and TopCount NXT Microplate Scintillation Counter (Packard Instruments, Meriden, CT), respectively. We tested 19 isolates from Myitkyina, 24 from Lashio, 12 from Mawlamyine, and 20 from Dawei (Table 1). In addition, 63 isolates from Thailand and 8 from Bangladesh were assayed for comparison.

TABLE 1

Summary of isolates collected from Myanmar, processed, successfully culture adapted, and tested by isotopic *in vitro* assays for antimalarial drug susceptibility

Isolate origin	Total collected	Contaminated	Culture adaptation: Attempted	Culture adaptation: Successful	Successful
Myitkyina	23	3	20	19	95% (19/20)
Lashio	31	3	28	24	86% (24/28)
Mawlamyine	33	16	17	12	71% (12/17)
Dawei	29	8	21	20	95% (20/21)
Total	116	30	86	75*	87% (75/86)

* Total isolates tested for *in vitro* susceptibility.

Parameter estimation. Concentrations that inhibit 50% (IC_{50}), 90% (IC_{90}), and 99% (IC_{99}) were estimated by nonlinear regression analysis of the concentration-response curve obtained from scintillometric data by a program originally developed by Desjardins and others[11] and modified for routine use at the Walter Reed Army Institute of Research, Department of Experimental Therapeutics (Kyle DE, unpublished data). Minimum inhibitory concentration (MIC), which is equivalent to IC_{99}, is the lowest concentration of drug at which no hypoxanthine incorporation is recorded. The MIC or cutoff concentration for the determination of *in vitro* resistance established at AFRIMS were 200 nM for chloroquine, 140 nM for mefloquine, and 2,000 nM for quinine. No MIC cutoffs for artemisinin compounds have yet been established.

Linear extrapolation of regression lines was made to allow for comparisons between 2 isolate groups on the basis of the log-probit analysis program designed for data obtained from morphology technique by means of World Health Organization-predosed *in vitro* assay plates.[12,13] At each drug concentration, inhibition of hypoxanthine incorporation was tested in duplicate. We calculated the average of the scintillation counts of the 2 wells and converted it into percentage of the average count of the control wells. The percentage data were then plugged into the log-probit program, from which parameters of the regression line and IC estimates at different drug concentrations were obtained. This allowed for the test of significance on the basis of potency ratio estimate for the comparison between 2 regression lines.[14] The relationship between IC_{50}s of different drugs was determined by Pearson correlation.

RESULTS

Overall, 76% (63 of 83) of the specimens from Lashio, Dawei, and Myitkyina were successfully recovered, culture adapted, and tested for *in vitro* susceptibility (Table 1). In

TABLE 2
Results of quinine *in vitro* susceptibility assays*

Isolate origin/yr	*n*	Geometric mean (95% confidence interval) IC_{50} (nM)	Geometric mean (95% confidence interval) IC_{90} (nM)	% *in vitro* resistance
Myitkyina/1998	19	212 (171–262)	457 (368–567)	15.8% (3/19)
Lashio/1997	24	221 (179–273)	519 (413–653)	20.8% (5/24)
Mawlamyine/1997	12	279 (224–347)	615 (507–746)	16.7% (2/12)
Dawei/1998	20	231 (176–302)	469 (364–604)	15.0% (3/20)
Maesod/1997	23	347 (281–427)	687 (571–826)	21.7% (5/23)
Sangkhlaburi/1996	19	273 (228–327)	682 (590–788)	47.4% (9/19)
Ranong/1996	11	262 (114–311)	576 (466–712)	27.3% (3/11)
Yala/1997	10	151 (122–188)	403 (316–514)	10.0% (1/10)
Chittagong/1997	8	283 (187–427)	539 (388–749)	0.0% (0/8)

* IC_{50} = concentration that inhibits 50%; IC_{90} = concentration that inhibits 90%.

Mawlamyine, unstable electricity interfered with our transport media storage and specimen processing procedures, resulting in 2 batches (totaling 16 specimens) being contaminated. Among the rest ($n = 17$), 12 (71%) were successfully culture adapted and tested. Average parasite density of the total 75 isolates that were subjected to *in vitro* microtests was 22,706/μL blood.

All except 2 isolates from Bangladesh, 3 from Lashio, and 2 from Dawei were *in vitro* resistant to chloroquine. The level of resistance was highest among the Thai isolates, followed by the isolates from Myanmar. In Thailand, the highest geometric mean IC_{50} and IC_{90} values for chloroquine was observed in Ranong, near the southernmost border to Myanmar (157 nM [95% confidence interval {CI}, 128–193] and 331 nM [95% CI, 262–419], respectively), and the lowest in Maesod. In Myanmar, geometric mean chloroquine IC_{50}s and IC_{90}s varied from 90 nM (95% CI, 75–109) and 176 nM (95% CI, 146–213) at Lashio to an IC_{50} of 132 nM (95% CI, 105–165) and IC_{90} of 242 nM (183–321) at Dawei. Isolates from Bangladesh were the least resistant, with geometric mean IC_{50} and IC_{90} of 88 nM (95% CI, 64–122) and 168 nM (95% CI, 118–237), respectively.

Quinine resistance in Myanmar was on average of slightly lower degree and prevalence than on the Thai side (Maesod, Sangkhlaburi, and Ranong). There were some variations in the IC_{50}s, IC_{90}s and MICs among the 4 Myanmar sites, but the highest geometric mean IC_{50} (279 nM [95% CI, 224–347]) and IC_{90} (15 nM [95% CI, 507–746]) were noted for Mawlamyine (Table 2).

The overall mefloquine *in vitro* susceptibility profile for Myanmar suggested a lower frequency of resistant isolates and a lower degree of resistance compared with Thailand, where the highest geometric mean IC_{90} of 172 nM (95% CI, 140–211) was estimated for Maesod, followed by Sangkhlaburi and Ranong (Table 3). Again, isolates from Mawlamyine were the most resistant among the 4 sites in Myanmar, with geometric mean IC_{90} of 124 nM (95% CI, 104–149), although its geometric mean IC_{50} of 51 nM (95% CI, 40–65) was far below that of Maesod (IC_{50} 92 nM [95% CI, 71–121]).

Figure 2 depicts a log-probit graph[13] comparing dose-response regression lines (obtained from the extrapolation of the radioisotope data) representing Maesod, Mawlamyine, and Lashio. Potency ratio estimates for the comparison between the regression lines of Maesod and Lashio as well as between Mawlamyine and Lashio indicated statistically significant differences in the response to mefloquine at $P < 0.05$. The isolates from Maesod were significantly less sensitive to mefloquine than the Lashio isolates. Similarly, the isolates from Mawlamyine were significantly less sensitive than those from Lashio. The *in vitro* sensitivity to artesunate varied only slightly across isolate origins. Mawlamyine and Lashio isolates had more elevated IC_{50}s and IC_{90}s than elsewhere (Table 4).

Pearson's correlation coefficients (r) and P values were estimated for the relationship between IC_{50}s (log scale) of different drugs among the 75 Myanmar isolates. Statistically significant correlation was noted between artesunate and mefloquine ($r = 0.31$, $P = 0.007$), artesunate and quinine ($r =$

TABLE 3
Results of mefloquine *in vitro* susceptibility assays*

Isolate origin/yr	*n*	Geometric mean (95% confidence interval) IC_{50} (nM)	Geometric mean (95% confidence interval) IC_{90} (nM)	% *in vitro* resistance
Myitkyina/1998	19	49 (37–65)	95 (76–119)	47.4% (9/19)
Lashio/1997	24	23 (17–29)	60 (46–77)	29.2% (7/24)
Mawlamyine/1997	12	51 (40–65)	124 (104–149)	83.3% (10/12)
Dawei/1998	20	36 (25–52)	75 (55–101)	40.0% (8/20)
Maesod/1997	23	92 (71–121)	172 (140–211)	95.7% (22/23)
Sangkhlaburi/1996	19	57 (44–73)	129 (100–167)	73.7% (14/19)
Ranong/1996	11	55 (39–79)	114 (87–148)	63.6% (8/11)
Yala/1997	10	21 (15–28)	54 (44–67)	20.0% (2/10)
Chittagong/1997	8	26 (18–39)	54 (42–69)	25.0% (2/8)

* IC_{50} = concentration that inhibits 50%; IC_{90} = concentration that inhibits 90%.

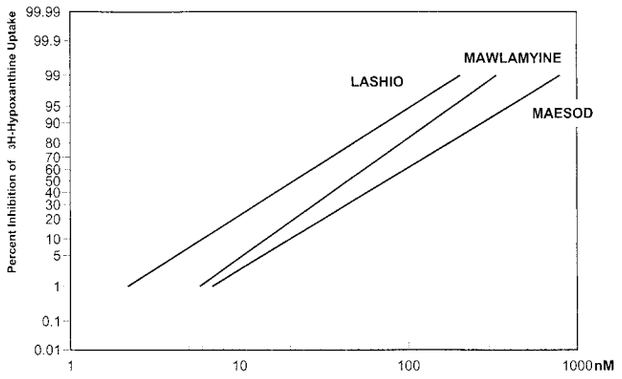

FIGURE 2. Log-probit regression lines (13) representing mefloquine *in vitro* susceptibility of *Plasmodium falciparum* from Maesod, Mawlamyine, and Lashio. If slope ratio (SR) is less than the factor of SR (fSR), the 2 lines are parallel within experimental error and the activities can be compared. If the potency ratio (PR) is greater than the factor of PR (fPR), the difference between the 2 lines is statistically different[14] at $P < 0.05$. Maesod versus Lashio: SR 1.05, fSR 1.52; PR 3.52, fPR 1.60 (statistically significant). Mawlamyine versus Lashio: SR 1.11, fSR 1.46; PR 2.09, fPR 1.66 (statistically significant). Maesod versus Mawlamyine: SR 1.16, fSR 1.55; PR 1.685, fPR 1.688 (not statistically significant).

0.26, $P = 0.02$), quinine and chloroquine ($r = 0.52$, $P = 0.0001$), and quinine and mefloquine ($r = 0.24$, $P = 0.04$).

DISCUSSION

Resistance of *P. falciparum* to various antimalarial drugs is a major obstacle to the control of malaria in Southeast Asia. In order to prevent the emergence and spread of multidrug-resistant malaria in this region, it is important that the status of antimalarial drug susceptibility be closely monitored. Although *in vitro* drug susceptibility surveillance of *P. falciparum* is routinely performed in Thailand, data from neighboring countries such as Myanmar, Cambodia, and Laos are scarce.

This study demonstrated a practical system for such a surveillance in remote, malarious areas, where poor public utilities, distance, and inconvenient transportation usually prohibit the establishment of an *in vitro* assay facility on site or collection of viable malaria parasites in order to transport them elsewhere. In spite of such difficulties, acquisition of malaria specimens for this study was accomplished through cooperation by all levels of the Myanmar health authorities. Specimen contamination rates (Table 1) were higher than expected. Nevertheless, the yield was satisfactory considering the field conditions encountered during specimen collection and processing. For our routine specimen collections in Thailand, strict sterile procedures were always attained and parasite recovery rate was > 90%.

Malaria continues to be endemic in most parts of Myanmar, with 79% of the total population of 47 million living in areas with malaria risk.[15] Although diagnosis is largely based on history and clinical findings, limited data based on blood smear examination indicated that 80.7% of the positive slides were *P. falciparum.* A nationwide estimate as of 1997 showed malaria morbidity rate to be 12.2 per 1,000 population, with Kayah State (bordering part of northwestern Thailand) and Chin State (on Myanmar's western borders with Bangladesh and India) having the highest rates (> 50 per 1,000 population). Average malaria mortality rate was 6.3 per 100,000 population nationwide, but a rate as high as 30.3 per 100,000 had been estimated for Kachin State.[15] Similar to most malaria-endemic countries, chloroquine resistance is widespread, but chloroquine is still commonly used because it is the most affordable therapy. Poor efficacy of sulfadoxine-pyrimethamine (S-P) in Myanmar was also recognized many years ago.[16]

TABLE 4
Results of artesunate *in vitro* susceptibility assays*

Isolate origin/yr	*n*	Geometric mean (95% confidence interval) IC_{50} (nM)	Geometric mean (95% confidence interval) IC_{90} (nM)
Myitkyina/1998	19	1.9 (1.6–2.3)	3.6 (2.8–4.5)
Lashio/1997	24	2.7 (2.0–3.6)	5.0 (3.8–6.7)
Mawlamyine/1997	12	3.2 (2.3–4.6)	5.4 (4.0–7.2)
Dawei/1998	20	1.8 (1.3–2.4)	3.4 (2.5–4.5)
Maesod/1997	23	2.7 (2.1–3.4)	4.4 (3.5–5.6)
Sangkhlaburi/1996	19	2.5 (1.9–3.3)	4.5 (3.7–5.6)
Ranong/1996	11	2.2 (1.4–3.4)	3.8 (2.6–5.5)
Yala/1997	10	1.5 (1.0–2.3)	3.2 (2.4–4.1)
Chittagong/1997	8	2.4 (1.8–3.2)	4.6 (3.3–6.4)

* IC_{50} = concentration that inhibits 50%; IC_{90} = concentration that inhibits 90%.

In Myanmar, *in vitro* chloroquine resistance was most marked at Myitkyina (Kachin State) and Dawei (Tanintharyi Division). This supported *in vivo* observations of lower chloroquine efficacy, 57% RII and RIII, in Tanintharyi Division than elsewhere in Myanmar.[15] The field efficacy of chloroquine in Myanmar, as well as in Bangladesh, suggests an important role of naturally acquired semi-immunity. This study did not include *in vitro* assays for sulfadoxine or pyrimethamine susceptibility, but a recent *in vivo* evaluation from Myanmar indicated poor clinical S-P efficacy on the basis of the 14-day test[17]—that is, 63% treatment failure in Tachileik, the Myanmar town near the northern part of the Thai-Myanmar border.[18] Our *in vitro* evidence and these limited *in vivo* findings confirm that chloroquine and S-P are no longer reliable for malaria treatment in Myanmar.

Percentages and levels of *in vitro* resistance to quinine among Myanmar isolates were on average below those observed in Thailand, except at Mawlamyine, where the geometric mean IC_{90} was close to that at nearby Maesod District (Thailand). Cross-resistance as a result of mefloquine pressure near the Thai border might have partially contributed to the high degree of quinine resistance in Mawlamyine. However, the data suggested that reduced quinine sensitivity existed all over Myanmar and was not confined to the border area.

The *in vitro* susceptibility profiles to mefloquine suggest that Mawlamyine is probably an area of transition between Thailand and inner Myanmar. In Thailand, mefloquine (750 mg single dose) is still operationally effective (i.e., field efficacy ≥ 70%) and is routinely used at outpatient malaria clinics outside of the high multidrug-resistance zones (mainly Maesod and the Thai-Cambodian border areas). It is therefore not surprising that regular-dose mefloquine (15 mg/kg) was highly effective in populations of Myanmar, with an average of 93% treatment success.[18] In Bangladesh, the most

recent available report also indicated high mefloquine efficacy.[19] Isolates from Yala, which is on the southern border of Thailand with Malaysia, indicate this to be an area where resistance to mefloquine and quinine is among the mildest in the country.

The high-level *in vitro* mefloquine resistance in areas on the Thai side of the Thai-Myanmar border such as Sangkhlaburi and Ranong seen in this study is quite alarming. Although mefloquine alone is still the operational drug for outpatients with falciparum malaria in these areas, its field efficacy will need to be reevaluated soon. Whether or not the high percentages of *in vitro* quinine resistance in Sangkhlaburi and Ranong (47.4 and 45.5%, respectively) were associated with quinine pressure due to hidden mefloquine failure needs to be further investigated. Similarly the unexplained elevation of geometric mean IC_{50} and IC_{90} of isolates from Myitkyina has to be followed closely.

Although no MIC cutoffs for *in vitro* resistance have been established for artemisinin drugs, the relatively low IC_{90}s are generally suggestive of continuing high sensitivity of falciparum isolates from this region. This study refuted an earlier concern that *P. falciparum* in Myanmar might have developed resistance to artemisinin derivatives. Although these drugs have been available without control in the country for more than a decade, their widespread consumption might have been hampered by the relatively high cost. Significance and clinical implications of the slightly raised artesunate IC_{50}s and IC_{90}s in Mawlamyine and Lashio are not known at present. *In vitro* monitoring efforts should therefore continue because the trend toward an increased use of artemisinin derivatives in this region is imminent.

A significant correlation between the IC_{50}s of mefloquine-quinine and artemisinin drugs was also observed in previous studies.[9,20] Earlier, this was thought to be the result of some overlapping in the mechanisms of action of the 2 drug groups rather than true cross-reactivity.[21,22] Recently, an experimental study of *pfmdr1* polymorphisms demonstrated that level of artemisinin sensitivity is modulated by mutations in Pgh1 (P-glycoprotein homologue 1 protein of *P. falciparum,* which is encoded by *pfmdr1* gene) and this *pfmdr1* effect parallels that observed with mefloquine in a strain-specific manner.[23] The epidemiological and pharmacodynamic significance of this phenomenon is not known.

In this study, we took advantage of the ability to cryopreserve *P. falciparum* isolates to obtain data that otherwise would not have been available. Adaptation of such isolates in culture before *in vitro* assays, however, is cause for concern because an isolate may comprise different parasite populations, and genotyping of *P. falciparum* before and after cultivation showed that alteration in the population composition occurred in ~ 70% of the isolates.[24] However, the parasite culture conditions used in our study, including the shorter than 6–8 weeks of cultivation duration reported by Viriyakosol and others,[24] probably do not contribute such significant changes to parasite subpopulations. These, plus the reasonable numbers of isolates accumulated, are likely to reflect the overall drug sensitivity profiles for each collection site. Also, comparison of the results among our study sites should be valid because of consistent use of cryopreserved specimens and the same culture adaptation technique. Application of this type of analysis to determine geographical variation of drug resistance and to predict trends in drug sensitivity patterns is a primary purpose of this study.

The results of this study and the poor clinical-parasitological efficacy of chloroquine and S-P[18] highlight the need for a revision of the antimalarial drug policy in Myanmar in order to reduce suffering and mortality from malaria. Such a drug policy will have to be in keeping with a realistic appreciation of epidemiological features, health service infrastructure, diagnostic and treatment potential, capability for vector control measures, and overall coverage of the control program. It should minimize the risk of occurrence and spread of drug resistance. However, the choice of suitable medicaments is limited as a result of the intensity of malaria transmission in various parts of Myanmar, an important constraint in using drugs with a long elimination half-life, such as mefloquine. Particularly difficult and challenging will be the development of a rational drug policy in areas of the Thai-Myanmar borders where multidrug resistance is already present. Here, an essential priority should be directed at delimiting the foci of mefloquine resistance and preventing the spread of resistance beyond these foci.

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