INTRODUCTION

Multidrug resistant malaria is a public health threat in Southeast Asia, and its potential spread is imminent. However, drug sensitivity reports from countries surrounding Thailand are sparse. To some extent, active malaria control efforts in countries like Thailand have prevented this problem from escalating in recent years. In Bangladesh, however, drug resistance has increased the burden of the country’s malaria control program. Up to 70,000 laboratory-confirmed and 900,000 clinical cases of malaria, with more than 500 deaths per year, were accounted for in Bangladesh in the late 1990s, but these numbers may represent a gross underestimate of the disease burden because of shortcomings in surveillance and information systems. Approximately 88% of the 125 million people in the country are at malaria risk.

The aim of this study is to characterize the dimension of in vitro antimalarial drug resistance in Bangladesh and to define baseline data for future assessments of drug susceptibility.

MATERIALS AND METHODS

Plasmodium falciparum isolates were collected in 1999 at the outpatient department of Ramu Health Complex (Coix’s Bazar district), Chittagong, Bangladesh, and the malaria clinic in Maesod, western Thailand. The samples were cryopreserved, culture-adapted, and tested for their drug susceptibility at the laboratory of the Armed Forces Research Institute of Medical Sciences in Bangkok using a [3H]-hypoxanthine uptake assay. The Thai samples, which represent isolates from a region with an exceptionally high prevalence of multidrug resistance, were gathered to serve as isolates from a region with an exceptionally high prevalence of multidrug resistance, as sexual forms per µL blood) were enrolled. Pregnant and lactating females, patients with severe malaria, those with a history of pretreatment, as well as young children were excluded. The samples were obtained before any treatments.

Patients subsequently received mefloquine (Lariam® 15 mg/kg single dose) for Bangladesh and artesunate-mefloquine combination (mefloquine 750 mg stat followed by 500 mg six hours later, plus artesunate 300 mg once per day for two days) for Thailand. Informed consent was obtained from all adult volunteers or from parents or legal guardians of minors. The study protocols were approved by the ethical review boards of Chittagong Medical College and the Thai Ministry of Public Health.

Inhibitory concentrations (ICs) were estimated by nonlinear regression analysis. For graphic display, the data were adapted to a log-probit model. Potency ratios (PR = IC50A/IC50B) were calculated as a measure of the different activity of antimalarial drugs at the two study sites. Geometric mean ICs were compared by Student’s t test.

RESULTS

Most of the P. falciparum isolates from Bangladesh tested (37/44; 84%) were found to be resistant to chloroquine (i.e., IC50 > 200 nM). The corresponding percentage was even higher in Thailand (95%, 21/22). Table 1 shows the geometric mean IC50 and IC90 values. With potency ratios (PR = IC50A/IC50B) were calculated as a measure of the different activity of antimalarial drugs at the two study sites. Geometric mean ICs were compared by Student’s t test.

Only 13 isolates from Bangladesh (30%) and five from Thailand (23%) were found to be resistant to quinine (i.e., IC50 > 2000 nM). The isolates from the Thailand-Myanmar border gave a higher geometric mean IC50 value; with a PR of 1.31, the difference was statistically significant at the IC50 level (P = 0.027).

The most notable difference was found for mefloquine. Twenty-seven of 44 isolates from Bangladesh (61%) and 18 of 22 from Thailand (82%) were in vitro resistant to mefloquine (i.e., IC50 > 200 nM). The geometric mean IC50 for the Thai isolates, however, was 1.6 times higher than that for the Bangladeshi isolates (PR = 1.64; P = 0.002). At the IC90 level, a similar relationship was found (PR = 1.31; P = 0.024). All Bangladeshi patients were clinically cured based on 28-day follow-up. Artesunate-mefloquine combination is the current first-line regimen for the study area in Thailand and, according to the Thai Malaria Control Program, the regimen continues to be fully effective in that population.
The geometric mean 50% and 90% inhibitory concentrations (GmIC50, GmIC90) with upper and lower 95% confidence intervals (UCI, LCI) for 

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<th>GmIC50 (nM)</th>
<th>GmIC90 (nM)</th>
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<tr>
<td></td>
<td>Mean</td>
<td>LCI</td>
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<tr>
<td>Bangladesh</td>
<td></td>
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<tr>
<td>Chloroquine</td>
<td>114.25</td>
<td>82.72</td>
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<tr>
<td>Quinine</td>
<td>291.52</td>
<td>233.20</td>
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<tr>
<td>Mefloquine</td>
<td>60.30</td>
<td>44.22</td>
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<tr>
<td>Artesunate</td>
<td>1.93</td>
<td>1.44</td>
</tr>
<tr>
<td>Thailand</td>
<td></td>
<td></td>
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<tr>
<td>Chloroquine</td>
<td>120.50</td>
<td>105.75</td>
</tr>
<tr>
<td>Quinine</td>
<td>382.34</td>
<td>312.88</td>
</tr>
<tr>
<td>Mefloquine</td>
<td>98.79</td>
<td>79.72</td>
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<tr>
<td>Artesunate</td>
<td>2.19</td>
<td>1.67</td>
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No in vitro resistance threshold has been defined for artemisinin compounds. However, the artemesine ICs for both Bangladesh and Thailand were of low levels and did not show any significant differences [IC50 (PR = 1.13; P > 0.05), IC90 (PR = 1.14; P > 0.05)].

Highly significant correlations were found between artemesine and mefloquine at EC50 (r = 0.651; P < 0.001) level. A similarly close relationship also was found between artemesine and quinine (r = 0.681; P < 0.001) as well as between mefloquine and quinine (r = 0.545; P < 0.001).

**DISCUSSION**

Our data suggest a moderate level of in vitro antimalarial drug resistance in Bangladesh. Chloroquine and artemesine susceptibilities show very little divergence between the two sites, but it is mefloquine that represents a key indicator of transition to a state of multirdrug resistance in Bangladesh.

Chloroquine resistance in Bangladesh has been known since 1976. The high levels of resistance found in this study are comparable to recent figures from Myanmar and the southern part of the Thailand-Myanmar border. The situation seems to have stabilized at a high degree of chloroquine resistance in Bangladesh as well as in Thailand.

The geometric mean IC50 for quinine of the parasites from Bangladesh was comparable to the one recently reported from Myanmar. Although significantly higher compared with Bangladesh, quinine IC50s for Thailand showed little change compared with previous years. The close relationship of quinine and mefloquine seems to have relatively modest impact on the long-term development of quinine resistance. To preserve its efficacy, especially for the treatment of severe malaria, quinine should be used judiciously, such as in combination with other antimalarials.

Although mefloquine has not been commonly used in Bangladesh, a large proportion of the isolates tested were found to be in vitro resistant to this drug. This may either be due to inherently mefloquine-resistant parasite populations or to the spread of resistant isolates from neighboring Myanmar. Our data, however, indicated that mefloquine was still considerably more active against *P. falciparum* in Bangladesh than in Thailand (Figure 1). It also should be noted that in vitro tests are independent of host immune response and must not be confused with clinical resistance. An earlier clinical study on the Myanmar-Bangladesh border suggested that mefloquine was still effective in that area. On the other hand, mefloquine IC50s for Bangladeshi isolates have already surpassed the levels noted for Myanmar. Assuming a progress of drug resistance in Bangladesh similar to the one in Thailand over a decade ago, a level of mefloquine resistance comparable to that observed on the western border of Thailand could be attained soon.

Our data indicated the continuing high sensitivity to artemesine of *P. falciparum* isolates from Bangladesh and Thailand. As in previous studies, a close activity correlation was observed between the artemesine ICs and those of mefloquine.

In conclusion, our data confirmed the loss of chloroquine sensitivity in Bangladesh. The surprisingly high prevalence of in vitro mefloquine resistance found in this study, together with the known resistance to sulfadoxine-pyrimethamine, may be a warning sign for substantial multidrug resistance and clinical mefloquine resistance in the future. As data from Bangladesh are still sparse, constant monitoring of antimalarial susceptibility and any attempt to avert potential problems associated with multidrug resistance are essential.

It should be noted that this type of in vitro assay data must be interpreted with caution. Our data provide a measure of antimalarial drug susceptibility in a way comparable to that of other similarly sampled and executed in vitro assessments. Trends in in vitro susceptibility to drugs by *P. falciparum* may reflect such trends in clinical treatment outcome. However, one set of in vitro assays per se does not provide a direct measurement of risk of therapeutic failure, especially in a semi-immune population.

**FIGURE 1.** Log-probit regressions for the mefloquine in vitro susceptibility in Bangladesh (Cox’s Bazar) and Thailand (Maesod). The distance between the two lines reflects a difference in regional mefloquine susceptibility.
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