Decreased In Vitro Susceptibility of Plasmodium falciparum Isolates to Artesunate, Mefloquine, Chloroquine, and Quinine in Cambodia from 2001 to 2007

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This study describes the results of in vitro antimalarial susceptibility assays and molecular polymorphisms of Plasmodium falciparum isolates from Cambodia. The samples were collected from patients enrolled in therapeutic efficacy studies (TES) conducted by the Cambodian National Malaria Control Program for the routine efficacy monitoring of artemisinin-based combination therapy (ACT) (artesunate-mefloquine and artemether-lumefantrine combinations). The isolates (n = 2,041) were obtained from nine sentinel sites during the years 2001 to 2007. Among these, 1,588 were examined for their in vitro susceptibilities to four antimalarials (artesunate, mefloquine, chloroquine, and quinine), and 851 isolates were genotyped for single nucleotide polymorphisms (SNPs). The geometric means of the 50% inhibitory concentrations (GMIC50s) of the four drugs tested were significantly higher for isolates from western Cambodia than for those from eastern Cambodia. GMIC50s for isolates from participants who failed artesunate-mefloquine therapy were significantly higher than those for patients who were cured (P, <0.001). In vitro correlation of artesunate with the other drugs was observed. The distributions of the SNPs differed between eastern and western Cambodia, suggesting different genetic backgrounds of the parasite populations in these two parts of the country. The GMIC50s of the four drugs tested increased significantly in eastern Cambodia during 2006 to 2007. These results are worrisome, because they may signal deterioration of the efficacy of artesunate-mefloquine beyond the Cambodian-Thai border.

Cambodia, especially the western border with Thailand, is known as a hot spot for multidrug-resistant malaria. Pyrimethamine resistance was first reported in that border area in the early 1950s (27), followed by chloroquine resistance in the late 1950s, sulfadoxine-pyrimethamine (SP) resistance in the late 1960s, and mefloquine resistance in the late 1980s (31). Since 1986 (5), the Cambodian National Malaria Control Program (CNMP) has set up a program to monitor the efficacy of SP and mefloquine monotherapy, specifically in the provinces bordering Thailand, Vietnam, and Laos. Between 1995 and 1999, failure rates based on 14-day follow-up periods were 7% to 30% for a low dose of mefloquine (15 mg/kg) and 7% to 10% for the higher doses (20 to 25 mg/kg of body weight) in provinces in northwest Cambodia bordering Thailand, while mefloquine efficacy remained 100% elsewhere (M. B. Denis, personal communication). These findings were consistent with reports from Thailand (21). In 2000, studies of the clinical efficacy of artesunate-mefloquine (AM) (12 mg/kg artesunate and 1,000 mg mefloquine given over 3 days) were conducted, again in northwestern Cambodia; they confirmed 100% efficacy based on a 14-day follow-up (M. B. Denis, personal communication). The NMCP decided to switch to AM combination therapy in the same year.

During an informal consultation on the monitoring of antimalarial drug efficacy in the Mekong Subregion organized by the World Health Organization (WHO) in Phnom Penh, Cambodia, in October 2000, it was agreed that each country should strengthen its capacity to monitor the efficacy of the first-line antimalarial drug. Monitoring should be conducted by therapeutic efficacy studies (TES) at selected sentinel sites on a regular basis, at least every other year for each site, with a 28-day follow-up. In 2001, the Center for Parasitology, Entomology, and Malaria Control of Cambodia (CNM) conducted TES in two provinces: Sampaoloum, in the northwestern part of the country near the Thai border, and Snoul, along the Vietnamese border. Both studies...
confirmed the high efficacy of AM, with cure rates of 96% and 100%, respectively (7).

In 2002, the failure rates of artemisinine-mefloquine increased to 14.3% in the northwest part of the country along the Thai-Cambodian border (7). In vitro monitoring conducted on isolates from Plasmodium falciparum-infected patients during the years 2001 and 2002 showed significantly higher geometric mean 50% inhibitory concentrations (GMIC50s) for mefloquine, chloroquine, and quinine in the western provinces than in the eastern provinces (13). The molecular marker of chloroquine resistance is also known to differ in its geographic distribution within Cambodia: the P. falciparum crt (pfcrt) CVIETIF/ISS haplotype was detected in 92% of isolates from the west but in only 11% of those from the east (14).

Since 2001, regular monitoring of the efficacy of artemisinin-based combination therapy (ACT), coupled with in vitro assessment and molecular marker assays, has continued uninterrupted. This article reports the temporal and geographic trends in the in vitro drug susceptibility assay results and the prevalence of single nucleotide polymorphisms (SNPs) in isolates from infected patients enrolled in the TES during the years 2001 to 2007. These findings were also analyzed in relation to the clinical outcomes of ACT.

MATERIALS AND METHODS

Sites and sampling. Fresh clinical isolates of P. falciparum were collected from patients participating in TES (also referred to as “in vivo studies” here) of AM and artemether plus lumefantrine (AL), conducted as part of the routine antimalarial drug efficacy monitoring of the NMCP. Venous blood (5 ml) was collected in Venoject tubes (Terumo Europe NV, Leuven, Belgium) containing EDTA and was transported to Phnom Penh within 48 h of collection at 4°C. Giemsa-stained thin blood smears were examined to determine parasite densities and to confirm P. falciparum monoinfection. When the parasitemia was between 0.1% and 1%, in vitro drug sensitivity tests were performed directly. If parasite density exceeded 1%, samples were diluted with uninfected erythrocytes (RBCs) to obtain an initial parasitemia between 0.5% and 1%. An aliquot of each isolate was frozen at −80°C for molecular analysis. If parasitemia recurred, an additional blood sample was collected onto 3M Whatman filter paper and was transported to the laboratory at room temperature and then kept at −20°C until DNA extraction.

TES. The monitoring of ACT efficacy in Cambodia by TES has been carried out by the CNM during 2001 to 2007 at 9 sentinel sites: Sampovloun, Veal Veng, and Pailin (western Cambodia), Preah Vihear and Anlong Veng (northern Cambodia), Ratanakiri and Snoul (eastern Cambodia), Chumkiri (southern Cambodia), and Oral (Kampot province, central Cambodia). To simplify our analyses, we divided the study locations into the eastern region, including sites in the north and the east (Preah Vihear, Anlong Veng, Ratanakiri, and Snoul), and the western region, including sites in the west, south, and center (Sampovloun, Veal Veng, Pailin, Chumkiri, and Oral) (Fig. 1).

These TES were performed according to the WHO 2003 protocol for low-transmission areas, and the clinical outcomes have been published previously (6, 7). Briefly, all patients over the age of 6 years and weighing >16 kg who presented with fever (defined as an axillary temperature of ≥37.5°C or a history of fever for 24 h prior to consultation) and had a smear positive for Plasmodium falciparum mono-infection with a density of 1,000 to 150,000 parasites/μl were included. Informed consent was given by the participant or by a parent/guardian for children. Enrolled patients had to stay at the hospital/health center until the 3-day AM or AL treatment regimen was completed and the blood smear became negative. They had to commit to a weekly follow-up for 4 weeks. Exclusion criteria were one or more of the general danger signs or any sign of severe and complicated malaria, pregnancy, febrile diseases other than malaria, severe malnutrition, and known hypersensitivity or contraindication to the study drugs.

For the AM group, a total dose of 12 mg/kg artemether and 25 mg/kg mefloquine was given over 3 days. The daily 4 mg/kg of artemether was divided into two equal doses, one in the morning and one in the evening, on day 0 and was given as a 4-mg/kg single dose on day 1 and day 2, with a maximum adult dose of 600 mg in total. Mefloquine (10 mg/kg) was also divided into two equal doses, one in the morning and one in the evening, on days 0 and 1, and was given as a 5-mg/kg dose once on day 2. The maximum dose of mefloquine was limited to 1,500 mg for adults, since side effects are common beyond this dose (7). For AL, participants received 20 mg/kg of artemether and 120 mg/kg of lumefantrine, or a total adult dose of 24 tablets (Coartem; Novartis, Switzerland), divided into two daily doses for 3 days. In the 2003 study, each dose of AL was provided with 250 ml milk and 5 pieces of coconut biscuit (6).

Participants were checked by blood smear for the presence of malaria parasites on study days 1, 2, 3, 7, 14, 21, and 28. If fever occurred at any time between

FIG. 1. Study sites for TES of drugs against P. falciparum malaria in Cambodia, 2001 to 2007.
the scheduled study days, an additional malaria smear was done. Participants who did not return according to schedule on their own were actively sought for. The therapeutic response was classified as early treatment failure (ETF), late clinical failure (LCF), late parasitological failure (LPF), or adequate clinical and parasitological response (ACPR) according to WHO protocols (29). Late treatment failure (LTF) includes LF and LPF and must be PCR corrected to exclude reinfection from the final data analysis (30).

In vitro susceptibility. (i) Preparation of drugs and test plates. Quinine hydrochloride was obtained from Sigma (Steinheim, Germany). Mel哲学, chloroquine diphosphate, and artesunate were obtained from RCC Ltd. via the Institut Pasteur in Paris, France. Stock solutions of chloroquine diphosphate and quinine were prepared in water (Biosedra, France), and stock solutions of mefloquine and artesunate were prepared in pure methanol. Subsequent 2-fold serial dilutions were prepared in distilled water (Biosedra, France). The final concentrations ranged from 0.05 to 51.2 nM for artesunate, from 1 to 1,024 nM for mefloquine, from 5 to 5,120 nM for chloroquine, and from 6.2 to 6,400 nM for quinine. Each concentration was used to coat two wells of a 96-well, flat-bottom Falcon plate (ATGC, France). Forty microliters of drug solutions, prepared as described above, was added to each well, and plates were then air dried in a laminar flow hood. The preloaded plates were stored at 4°C until use. Test plates were prepared weekly and were used within 2 weeks after preparation. Their suitability for in vitro testing was regularly monitored using reference strain 3D7, maintained in continuous culture and presenting known responses to the various drugs tested.

(ii) Isotopic assay. The sensitivities of the Cambodian isolates to drugs were assessed in vitro by the use of a classical isotopic (48-h) test (8). Briefly, fresh blood samples were washed three times with RPMI 1640 medium (Gibco, In vitro Technologies Corporation, France), followed by centrifugation (800 × g, 10 min). The parasites were then tested directly without culture adaptation. Infected erythrocytes (1.5% hematocrit; 0.1% to 1% parasitemia) were suspended in complete RPMI medium supplemented with 10% AB positive human serum inactivated for 30 min at 56°C (Biomedia, France) and buffered with 25 mM HEPES and 25 mM NaHCO3. The mixture was transferred (200 μl per well) to the 96-well test plates that had been precoated with antimarial agents. Each plate included two drug-free control wells and one control well without parasites. The culture plates were incubated for 48 h at 37°C in a candle jar. [3H]hypoxanthine (0.5 μCi/well; Amershams, Biosciences, France) was used to assess parasite growth. Each isolate was tested in duplicate in microplates with serial dilutions of drugs. Drug response was quantified by measuring [3H]hypoxanthine uptake in a Wallac MicroBeta TriLux counter (Perkin-Elmer, France). At the end of the incubation period, the plates were frozen at −20°C and were thawed to lyse the cells. After collection on glass fiber filter paper using a cell harvester, the amount of [3H]hypoxanthine incorporated into parasite nucleic acids was determined. The results of the in vitro assay are expressed as the 50% inhibitory concentration (IC50), defined as the concentration at which the incorporation of [3H]hypoxanthine was inhibited 50% relative to that in the drug-free control wells. Parasite growth was measured by using a log-probit approximation to determine the IC50.

Drug sensitivity tests. (i) DNA extraction. Parasite DNA was extracted from frozen blood aliquots (200 μl) using a High Pure PCR template preparation kit (Roche Diagnostics Gmbh, Mannheim, Germany) according to the manufacturer’s protocol. Blood stored on filter paper was extracted using a QIAamp DNA minikit (Qiagen GmbH, Hilden, Germany) as previously described (11, 22).

(ii) Genetic polymorphism. Paired samples taken at enrollment and at recurrence of parasitemia were used to distinguish between recrudescence and reinfection. The number of variants in each of three polymorphic genes (mspl, mspl2, and glurp) was determined as described previously (11, 25). If the sample taken at recurrence contained either a subset of, or the same variants as, those in the enrollment specimen, the infection was classified as a recrudescence. If not, the infection was classified as a reinfection. The amplification of a single fragment at each of these three loci indicated that the parasite population was monoinfected (a single genotype). Detection of two or more PCR bands at one or more loci indicated that the isolate contained multiple genotypes.

Nine SNPs of four genes—pfmdr1 codons 511L, 519R, 5108(N/T), and 1164L, pfet7 codon K76T, pfmdr1 codons N86Y, Y184F, and N1042D, and pflaspe6 codon 5769N—were analyzed using a microarray-based assay as described previously (4). Cutoff values were determined by an algorithm. Fluorescence intensities below 9,000 U (Cy3) or 10,000 U (Cy5) (mean intensities minus background) were regarded as negative. For measures above these cutoff values, we considered the ratio of Cy5 intensity to Cy3 intensity to discriminate between wild type or mutant (a mixed signal was classified as mutant). Controls included previously sequenced samples and strain 3D7.

Statistical analysis. All statistical analyses were performed using Stata/SE, version 8 (Stata Corporation, College Station, TX). Information on location, age, sex, parasitemia, and clinical outcome was collected in an anonymous database. The in vitro activity of each antimalarial was expressed as the geometric mean of the IC50 for all isolates. Drug concentrations were transformed into logarithms. The Wilcoxon rank-sum (Mann-Whitney) test was used to determine whether the observed differences in the in vitro responses to antimalarial drugs were significant. Linear regression analysis was used to assess the relationship between age, sex, parasitemia, and pfmdr1 polymorphisms. The level of significance was adjusted using the Bonferroni correction. For all statistical tests, the significance level was set at a P value of 0.05.

Ethics. The study was approved by the National Ethics Committee for Health Research of the Cambodian Ministry of Health, the Institutional Review Board of the Naval Medical Research Unit No. 2, and the Technical Review Group of WHO/WPRO.

RESULTS

A total of 2,041 P. falciparum isolates were collected during the years 2001 to 2007. The geographical locations of the collection sites and the number of isolates collected per year per site are shown in Fig. 1 and Table 1, respectively. Because of the discrepancy in the efficacy of AM between areas of endemism in eastern and western Cambodia (7), further analyses were done based on the grouping of isolates into eastern and western Cambodia. Most of the data collected in 2001 and 2002, published previously (13), are also included to serve as baseline values. This set of older data was reanalyzed and standardized with the data from 2003 to 2007.

There were marked differences in the study subject demographics between the eastern and western sites. The median age was lower (17 versus 22 years; P <0.001 by the Wilcoxon rank-sum [Mann-Whitney] test), and the proportion of male participants was lower (0.6 versus 0.7; P <0.001 by the χ2 test) in the east than in the west (Fig. 2). However, the geometric mean parasitemia was slightly higher for the west than for the east (0.28% versus 0.23%; P =0.001 by the Wilcoxon rank-sum [Mann-Whitney] test). As expected, participants from eastern Cambodia responded to AM better than those from western Cambodia (ACPR, 98.7% versus 89.9%; P <0.001 by the χ2 test).

Out of the 2,041 isolates, 1,588 (78%) were examined for their in vitro sensitivities to four antimalarial drugs; 274 isolates were excluded because of low parasitemia (<0.1%). An additional 179 isolates were not analyzed because of delayed receipt of samples at the Institut Pasteur in Phnom Penh. Of the isolates analyzed, 820 (52%) gave interpretable results for at least one of the drugs tested. The fact that we did not attain a better success rate for the in vitro assays may be due to rela-
tively low initial parasitemias (<0.2% for 778 isolates), suboptimal storage conditions, complications in the transportation of samples, or the possible presence of trace drug in the samples (although RBCs were washed three times before culture).

The overall geometric means of IC₅₀ (GMIC₅₀) are presented in Table 2. No significant correlation was found between IC₅₀ and sex, age, or parasitemia for any of the drugs tested. No attempt was made to test for the presence of antimarial drugs in the blood samples. The GMIC₅₀s of all four tested. No attempt was made to test for the presence of anti-malarial drugs in the blood samples. The GMIC₅₀s of all four drugs tested were significantly higher in the western than in the eastern provinces (P < 0.001) (Table 3).

There were important differences in the GMIC₅₀ over time during the study period (2001 to 2007). The GMIC₅₀ for artesunate began to rise in the west in 2004 but remained low in the east until 2007, when it increased significantly (Table 4). Similarly, the GMIC₅₀ for mefloquine began to increase in the west in 2004 but did not increase significantly in the east until 2007 (Table 5). The GMIC₅₀s for chloroquine and quinine have increased in both the western and eastern provinces since 2004, but the GMIC₅₀ for both drugs maintained their higher levels in the west (Tables 6 and 7).

We next compared the GMIC₅₀ of the four tested drugs with clinical outcomes following AM treatment (Table 8). The results show that for all drugs tested, the GMIC₅₀ were significantly higher for patients with LTF than for patients who were cured. No cutoff value was applied to the in vitro IC₅₀ level in order to determine its association with therapeutic failure.

There was a significantly positive correlation between the in vitro activity of artesunate and those of the other three drugs: mefloquine (Pearson’s r, 0.338; n = 755; P < 0.001), chloroquine (Pearson’s r, 0.327; n = 770; P, 0.001), and quinine (Pearson’s r, 0.340; n = 738; P < 0.001). The correlations were not significantly different when analyzed separately for eastern and western Cambodia.

A total of 851 isolates from patients enrolled during 2003 to 2006 were randomly selected for microarray analysis of polymorphism in four genes (pfldhfr, pfcrf, pfmdr1, and pfatpase6). Interpretable results ranged from 52.4% for pfmdr1 N1042D to 92.8% for pfmdr1 N86Y. Table 9 summarizes the microarray results. Of the 9 SNPs tested, 7 showed mutation frequencies greater than 5% (Fig. 3). Of these, we observed 3 SNPs that showed significantly different distributions in eastern versus western Cambodia (pfldhfr N51I and I164L and pfmdr1 Y184F).

We did not find any SNPs showing a significant association...
with the year, parasitemia, age, sex, or the clinical outcome following AM therapy, pfmdr1 codon Y124F and pfldhfr codon I64L was found to be associated with an increased chloroquine GMIC50, and pfdhfr codon Y184F was found to be associated with an increased chloroquine GMIC50 (n = 113; P = 0.003), but only for eastern Cambodia.

**DISCUSSION**

There has been a major reduction in the number of *P. falciparum* malaria cases in Cambodia for the past decade. The number of registered cases dropped from 130,000 in 2000 to 60,000 in 2007. Malaria control was particularly successful along the Cambodian-Thai border, and the malaria prevalence in that area is currently very low, an observation supported by the small number of isolates showing multiple genotypes. More than 82% of the isolates contained only one parasite population in that area is currently very low, an observation supported by the small number of isolates showing multiple genotypes. More than 82% of the isolates contained only one parasite population. With the year, parasitemia, age, sex, or the clinical outcome following AM therapy.
drug resistance, we believe that it is important to include these findings. Interestingly, despite the lower geometric mean artesunate and mefloquine IC50s for Chumkiri than for other western sites, a high AM recrudescence rate (13.1% by day 28) was observed there (19). In addition, for isolates obtained from participants who suffered recrudescence, the mean IC50s of both mefloquine and artesunate were higher than those for participants who did not suffer recrudescence.

Previous studies showed that pfmdr1 copy numbers and the prevalence of the mutation in pfmdr1 codon 184 were higher in western than in eastern Cambodia (23). Analysis of pfmdr1 copy numbers was also conducted in our laboratory and was reported previously (12). According to that report, the correlation between increased pfmdr1 copy numbers and increased mefloquine IC50s was statistically significant. However, we did not find a statistically significant correlation between increased pfmdr1 copy numbers and increased artesunate IC50s. Although the relationship between the pfmdr1 copy number and in vitro sensitivity to artesunate has been shown experimentally (24), field studies do not always demonstrate that relationship (9, 19), raising the suspicion that current in vitro assay methods may not be sensitive enough to detect a change in artemisinin susceptibility levels in the field.

We confirm here the higher frequency of the pfmdr1 184F mutant in western Cambodia. Recent studies have identified an association between pfmdr1 point mutations and decreased in vitro susceptibility to other antimalarials, including mefloquine, halofantrine, quinine, and artemisinin (10, 20, 26, 32). The pfmdr1 codon 184 mutant was associated with an increased mefloquine IC50 (16, 17). It will be worthwhile to further explore the significance of the pfmdr1 184F mutation as a marker of mefloquine resistance.

The striking difference in the prevalence of the pfldhfr codon 164 mutant between eastern and western Cambodia (12.1% versus 73.8%; P < 0.001 by the χ² test) is of interest. Although sulfadoxine-pyrimethamine no longer has any therapeutic value in this area of malaria endemicity, a follow-up to see if any changes occur over time with this marker may be epidemiologically important. The SNP data presented here may also serve as a baseline for the monitoring of some antimalarials to be deployed in the future.

The various SNPs found in this study correlated neither with the clinical outcome nor with the in vitro IC50 of any tested drug, but their distinct geographic distribution suggests different genetic makeup of malaria parasites in the eastern and the western areas of endemicity. The different allelic distributions could be the result of selection and/or a drift acting on semi-isolated parasite subpopulations (1). Indeed, the areas are geographically separated by the natural barrier of the Tonlé Sap channel, where rice fields are not suitable for the ecology of Anopheles (Fig. 1). However, we are aware that these preliminary conclusions need to be substantiated with a robust population genetics study.

The positive correlation detected among the antimalarial drugs, including amino alcohols, is consistent with previous reports (3, 13, 18), thus reflecting an alarming multidrug-resis-

### TABLE 8. Comparison of in vitro GMIC50s for *P. falciparum* isolates in the ACPR and LTF groups of the TES (28-day follow up) by drug

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of isolates</th>
<th>GMIC50 (range) (nM)</th>
<th>No. of isolates</th>
<th>GMIC50 (range) (nM)</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artesunate</td>
<td>558</td>
<td>0.83 (0.05–8)</td>
<td>37</td>
<td>1.72 (0.4–7.1)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Mefloquine</td>
<td>530</td>
<td>23.65 (1–181)</td>
<td>35</td>
<td>50.57 (6.8–142.4)</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>551</td>
<td>139.15 (7.16–814)</td>
<td>36</td>
<td>179.43 (46.2–650.6)</td>
<td>0.0192</td>
</tr>
<tr>
<td>Quinine</td>
<td>516</td>
<td>113.03 (10.3–715.6)</td>
<td>36</td>
<td>201.14 (53.8–698.4)</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

*Comparisons were tested by the Wilcoxon rank-sum (Mann-Whitney) test.

### TABLE 9. Number and frequency of microarray-based SNP assays performed and mutants detected disaggregated by gene and specific point mutation

<table>
<thead>
<tr>
<th>Gene and codon change</th>
<th>No. of analyses (% of isolates)*</th>
<th>No. of mutations (frequency %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>dhfr</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N51I</td>
<td>774 (91)</td>
<td>688 (88.9)</td>
</tr>
<tr>
<td>C59R</td>
<td>754 (88.6)</td>
<td>731 (96.9)</td>
</tr>
<tr>
<td>S108(N/T)</td>
<td>580 (68.2)</td>
<td>571 (98.5)</td>
</tr>
<tr>
<td>I164L</td>
<td>644 (75.7)</td>
<td>307 (47.7)</td>
</tr>
<tr>
<td>pfCRT (K76T)</td>
<td>768 (90.2)</td>
<td>764 (99.5)</td>
</tr>
<tr>
<td>pfmdr1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>N86Y</td>
<td>790 (92.8)</td>
<td>5 (0.6)</td>
</tr>
<tr>
<td>Y184F</td>
<td>639 (75.1)</td>
<td>352 (55.1)</td>
</tr>
<tr>
<td>N1042D</td>
<td>446 (52.4)</td>
<td>38 (8.5)</td>
</tr>
<tr>
<td>pfATPase6 (S769N)</td>
<td>547 (64.3)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

*a A total of 851 isolates from the patients enrolled were selected for a microarray-based SNP test.

![FIG. 3. Distribution of *P. falciparum* drug resistance SNPs in Cambodia.](image-url)

p<0.001

West □ East
TABLE 10. GMIC\textsubscript{50} for the P. falciparum 3D7 clone by the \textit{in vitro} susceptibility assay (2001 to 2007)\textsuperscript{a}  

<table>
<thead>
<tr>
<th>Drug</th>
<th>No. of isolates</th>
<th>Mean GMIC\textsubscript{50} (nM) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Artesunate</td>
<td>38</td>
<td>0.9 (0.7–1.07)</td>
</tr>
<tr>
<td>Mefloquine</td>
<td>37</td>
<td>34.5 (27.7–43.02)</td>
</tr>
<tr>
<td>Chloroquine</td>
<td>35</td>
<td>18.7 (16.45–21.19)</td>
</tr>
<tr>
<td>Quinine</td>
<td>33</td>
<td>59.9 (45.33–79.22)</td>
</tr>
</tbody>
</table>

\textsuperscript{a} The 3D7 clone was tested for purposes of quality control. These tests were carried out several times a year to control the batch plates.  
\textsuperscript{b} 95\% CI, 95\% confidence interval.

SUSCEPTIBILITY OF \textit{P. Falciparum} TO ANTIMALARIALS 2141

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95\% CI, 95\% confidence interval."


