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Author(s)	Hatabu, Toshimitsu; Vanisaveth, Viengxay; Taguchi, Nao; Kobayashi, Jun; Mannoor, M.Kaiissar; Watanabe, Hisami; Toma, Hiromu; Phompida, Samlane; Kano, Shigeyuki
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A PILOT FIELD SURVEY ON THE *IN VITRO* DRUG SUSCEPTIBILITY OF *PLASMODIUM FALCIPARUM* IN LAO PDR

TOSHIMITSU HATABU[1,2], VIENGXAY VANISAVETH[3], NAO TAGUCHI[1], JUN KOBAYASHI[4,5], M. KAIISSAR MANNOOR[6], HISAMI WATANABE[7], HIROMU TOMA[6], SAMLANE PHOMPIDA[3], and SHIGEYUKI KANO[2,*]

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In Southeast Asia, malaria has presented a major public health problem, and the spread of drug-resistant falciparum malaria is making the problem more serious in this region. Thus, evidence-based detection of drug-resistant parasites is important for the accurate evaluation of susceptibility to antimalarial drugs. Lao PDR (Lao People's Democratic Republic) is a developing country in which about 70% of the population lives in malaria endemic areas. Because of the lack of information on the *in vitro* drug susceptibility of parasites in this country, chloroquine (CQ) is still the drug of choice for uncomplicated falciparum malaria [1]. This report is a pilot field survey on the *in vitro* CQ- and mefloquine (MQ)-susceptibility of falciparum malaria using AnaeroPack® gas system in Saravan province, Lao PDR.

Saravan province is located in the southern part of Lao PDR. The survey in this province was conducted from August 8 to 16, 2003. Blood samples were successfully obtained from nine Laotian patients suffering from falciparum malaria. The samples were collected by the staff of the Center of Malariology, Parasitology and Entomology, after explaining the purpose of the study to the patients. The survey was conducted in accordance with the ethical guidelines for epidemiological studies established by the Ministry of Education, Culture, Sports, Science and Technology and Ministry of Health, Labour and Welfare of Japan. The *in vitro* drug susceptibility test was administered using the AnaeroPack® malaria culture system with a thermostat portable incubator as described previously [2, 3]. The AnaeroPack® CO_2 (Mitsubishi Gas Co., Tokyo, Japan) is a foil-packed paper sachet that on exposure to air immediately absorbs atmospheric O_2 and simultaneously generates CO_2 until a condition of 15% O_2 and 5% CO_2 is attained. The microaerophilic atmosphere produced within a sealed jar (AnaeroPack® Kakugata jar, SUGIYAMA-GEN Co., Ltd., Tokyo, Japan) can be maintained for at least 24 hours. The temperature inside the portable thermostat incubator (SUGIYAMA-GEN Co., Ltd.) was adjusted to 37 ℃. During *P. falciparum* cultivation, the sachet inside the jar was replaced every day when the culture medium was changed. The WHO semi-micro test method was used for evaluation of *in vitro* drug susceptibility [4]. Briefly, blood samples (0.1 ml) were resuspended in RPMI 1640 (GIBCO BRL), pH 7.4, supplemented with 25 mM HEPES, and sodium bicarbonate. To monitor parasite growth, six wells per plate served as controls without antimalarials. When the schizonts were fully grown in the control wells, the culture plate was removed from the incubator. Thin-smear specimens stained with Giemsa solution were made from each well. We defined parasites as schizonts when they had both dark brown pigment and more than three nuclei [5]. The effect of antimalarials on parasite growth was evaluated by the WHO standard evaluation method.

The results of this study are shown in Table 1. When complete schizont inhibition is observed at a CQ amount of

1 Gunma University School of Health Sciences, Gunma, Japan;
2 Research Institute, International Medical Center of Japan, Tokyo, Japan;
3 Center of Malariology, Parasitology and Entomology, Vientiane, Lao PDR;
4 Asian Centre of International Parasite Control, Bangkok, Thailand;
5 Bureau of International Cooperation, International Medical Center of Japan, Tokyo, Japan;
6 Division of Tropical Parasitology, Faculty of Medicine, University of the Ryukyus, Okinawa, Japan;
7 Division of Cellular and Molecular Immunology, Center of Molecular Biosciences, University of the Ryukyus, Okinawa, Japan.
* Correspondence:
Shigeyuki Kano,
Research Institute, International Medical Center of Japan
1-21-1 Toyama, Shinjuku, Tokyo 162-8655, Japan
TEL: +81-3-3202-7181 (ext 2877), FAX: +81-3-3202-7287, E-mail: kano@ri.imcj.go.jp

Table 1: The results for *in vitro* drug susceptibility

No.	Parasitemia (%)	Chloroquine	Mefloquine
A	0.015	Susceptible	Susceptible
B	0.36	Susceptible	Susceptible
C	1.97	Susceptible	Susceptible
D	0.91	**Resistant**	Susceptible
E	0.01	**Resistant**	Susceptible
F	0.13	**Resistant**	Susceptible
G	0.002	**Resistant**	Susceptible
H	0.004	Susceptible	Susceptible
I	0.007	Susceptible	Susceptible

80 nM or less, the parasite is considered susceptible. If schizont formulation is observed at an MQ amount of 640 nM or more, the parasite can be considered resistant. In the present study, four (44%) of the nine isolates were resistant to CQ, while all the isolates were susceptible to MQ. There was no correlation between the parasitemia and CQ-resistance.

The results of this study suggest that CQ-resistant parasites have increased even though CQ is commonly used as the first-line drug for treatment of uncomplicated falciparum malaria in Lao PDR. In neighboring countries such as Thailand and Cambodia, high-grade multi-drug resistant parasites are reported to be spreading and, indeed, *in vivo* CQ-resistant falciparum malaria has already been reported in Lao PDR [6]. Dedicated efforts have to be made to determine the *in vitro* drug susceptibility of *P. falciparum* in Lao PDR as a way to prevent the spread of multi-drug resistant parasites in the near future. This is the first test report on *in vitro* drug resistance in Lao PDR.

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