DRUG SUSCEPTIBILITY OF *PLASMODIUM FALCIPARUM* COLLECTED FROM DIFFERENT AREAS OF THAILAND DURING 2000 – 2001

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Abstract

Eighteen isolates of *Plasmodium falciparum* isolates were collected from (1) Ratchaburi, (2) Tak, (3) Ranong, and (4) Ubon Ratchathani. Only sixteen isolates were successfully adapted to our laboratory environment. All isolates were tested against 5 antimalarial drugs, pyrimethamine, chloroquine, quinine, mefloquine and amodiaquine. The range of MIC against all tested drugs are similar to those reported earlier. However, the falciparum isolates from Ubon Ratchathani tend to be more sensitive than isolates from other provinces. This suggested that the malaria parasites from Thai-Laos border and Thai-Myanmar border may have different genetic background or may be selected with drug pressure at different rates.

keywords: Plasmodium falciparum, drug susceptibility, pyrimethamine, chloroquine, quinine, mefloquine, amodiaquine

Introduction

Malaria is a parasitic disease that infects millions of human worldwide. In Thailand, although special task forces and budgets had been allocated to fight with the disease, more than 30,000 malarial cases had been reported in 2006 by Malaria cluster, Department of Disease Control, Ministry of Public Health[1, 2].

During extensive battle against malaria, several drug regimens have been changed as the parasites developed themselves into drug resistance organisms. Several *P. falciparum* isolates have been reported to resist against quinine, pyrimethamine, chloroquine and mefloquine around the globe. In Thailand, most of the falciparum parasites show little or no response to different drug combinations, such as pyrimethamine-sulfadoxine or pyrimethamine-sulfadoxine-mefloquine[3].

To allow the policy maker to dynamically

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adjust or change the drug regimen, the data of drug susceptibility against malarial drugs both old and new must be monitored periodically. The drug susceptibility data against the old drugs will help us to learn their resistant status while the data of the using drug will help us evaluate the effectiveness of the current treatment.

The aim of this project is to collect *P. falciparum* from different parts of Thailand and test for their drug susceptibility *in vitro* which will help us to determine drug susceptibility under condition free from environment, behaviour and genetic factor interference as found *in vivo* test.

Materials and methods

Parasite Collection

P. falciparum samples were received from Malaria clinics in 4 different provinces of Thailand, namely, (1) Ratchaburi and (2) Tak located in western part of Thailand, (3) Ranong, located in southern part of Thailand and (4) Ubon Ratchathani, located in eastern part of Thailand (Fig. 1). All samples were mixed with transferred medium which contains complete RPMI medium supplement with 10% serum and transferred to Bangkok on the same day in an ice box[4]. These isolates were named according to their origins; RB (from Ratchaburi), RN (from Ranong) and UB (from Ubon Ratchathani).

Parasite Cultivation

At their arrival, the parasites were all cultured in the laboratory by the candle-jar method[5]. The isolates which show no sign of growth or contaminated with bacteria or yeast within 1 month of cultivation were discarded. Culture adapted isolates were periodically checked for their growth and morphology using thin smear and stained with Giemsa's stain.

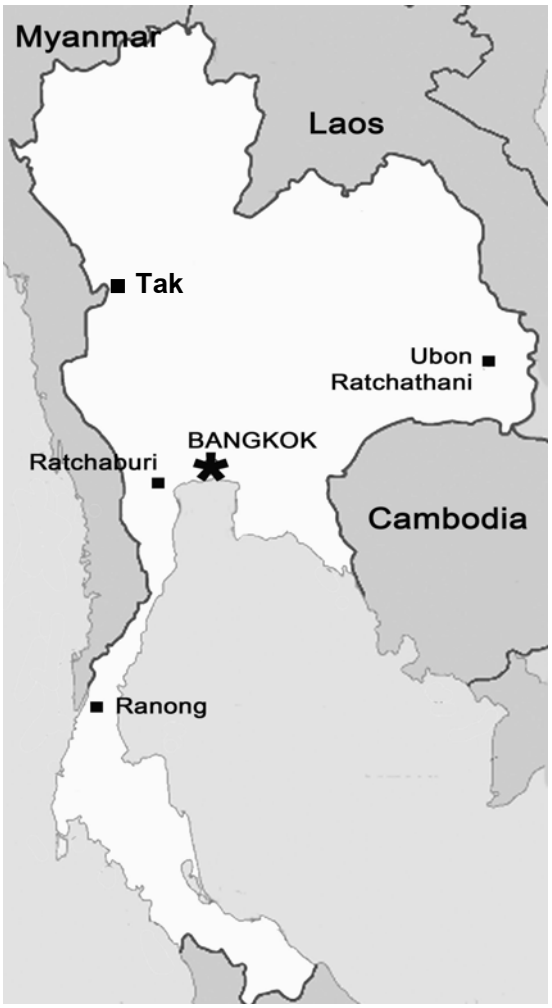


Figure 1 The location of 3 provinces where the malaria parasites were collected. The parasites were marked as RB, T, RN and UB according to their origins, Ratchaburi, Tak, Ranong and Ubon Ratchathani respectively.

Drug Susceptibility Test

Six malaria drugs were subjected to test against all isolates; Pyrimethamine (Sigma), Chloroquine diphosphate (Resochin, Bayer), Quinine sulphate (Roche), Amodiaquine hydroxide (Roche), Mefloquine hydrochloride (Roche) and Artemisinine (kindly provided by Prof. Kesara Na-Bangchang). To determine susceptibility against each malaria drugs, 100 μl of complete RPMI medium containing 10% serum and different drug concentrations were added

into each well of 96-well culture plate[6]. Each drug concentration were prepared in duplicate wells. Before the test were performed, *in vitro* adapted isolates were propagated, some of which have been cryopreserved in liquid nitrogen for further study. Each isolates were added into medium-filled wells described above at the initial parasitaemia between 0.3 - 0.8% with 5% haematocrit. To maintain the parasite growth, the plates were processed as normal cultivation method. Fresh medium with or without drug must be replaced at 24-hour interval. After 72 hours of incubation, the lowest drug concentration which kills all or nearly all parasites or Minimum Inhibitory Concentration (MIC) value of each isolates were determined by thin film investigation. Clone, T9/94RC17, were used as a control because its MIC value has been stable since its recloning by micromanipulation method.

Results and Discussion

Eighteen isolates were received from Malaria Clinics in 4 provinces of Thailand however, only sixteen isolates were successfully cultured *in vitro* (Table 1). Three major factors accounted for the small number of collected isolates, (1) the reduction of malaria-infected cases, (2) only a two-days trip was operated at each Malaria Clinics to allow the establishment of parasite culture on the same day of collection, (3) fungi contamination. The reduction of malaria patients seems to be a major impact on the number of collectable isolates. In the past few years, malaria infection was limited at around 30,000 cases per year compared with approximately 100,000 cases in 1997[2]. As a result, a small number of patients were available for the parasite collection during our field trips.

Table 1 The number of isolates which were received from Malaria Clinics in 4 provinces of Thailand during 2000 - 2001.

Provinces	Received isolates	Established isolates
Ratchaburi	6	RB1, RB2, RB3 RB4, RB5, RB6
Ranong	6*	RN1, RN2 RN4, RN5
Ubon Ratchathani	4	UB1, UB2 UB3, UB4
Tak	2	T137, T138

*RN3 and RN6 were contaminated with fungi and were discarded.

The drug susceptibility results revealed that these isolates are still highly resistance to pyrimethamine (Fig 2). The parasites which have the highest MIC can be found only in samples collected from Tak and Ratchaburi. All samples from Ubon Ratchathani have the lowest MIC in this experiment, $5X10^{-5}$ M. In comparison to 10^{-9} M, the lowest MIC found in pyrimethamine-sensitive isolates[7], the malaria parasites have maintains their resistance status from 1979 up to now[8].

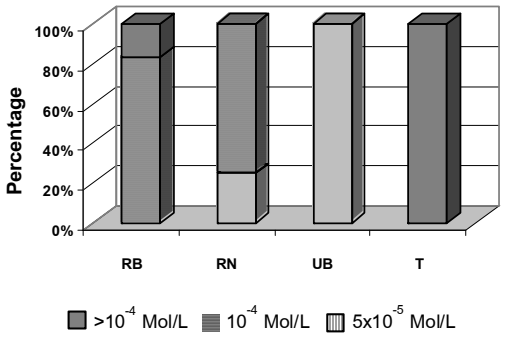


Figure 2 Drug susceptibility against pyrimethamine of all isolates from different parts of Thailand. Each colored bar represents a proportion of each MIC value in percentage. The MIC is reported as Molar concentration.

Similarly, the parasites collected during 2000 – 2001 are highly resistance to chloroquine (Fig 3). The isolates which have the highest MIC value are in Ratchaburi, 10^{-6} M, the highest MIC value reported previously[7]. Surprisingly, an isolate from Ubon Ratchathani show the lowest MIC value, $5X10^{-8}$ M, ever reported. This isolate should be marked as chloroquine-sensitive isolate. Genetic study of this isolate may help to reveal genetic mechanism related to chloroquine resistance.

The drug susceptibility analysis of the collected isolates shows that the quinine (Fig 4) and amodiaquine (Fig 5) sensitivity has not changed from the past[7]. The highest quinine-MIC parasites are found along Thai-Myanmar border, Tak, Ratchaburi and Ranong while the lowest quinine-MIC parasites are found in all provinces except Tak. Again, all parasites from Ubon Ratchathani show the lowest quinine-MIC in this experiment. Because quinine is currently using as a malaria treatment in combination with an antibiotic, e.g. tetracycline or doxycycline, in Thai hospital, the decreasing of quinine sensitivity could be expected in the future.

Malaria isolates in this study showed different susceptibility against mefloquine (Fig 6), used alone or in combination with artesunate by malaria clinics. Similar to pyrimethamine testing result, the parasites which show highest MIC are found in Ratchaburi and Tak while the parasites with lowest MIC are found in Ratchaburi and, again, Ubon Ratchathani. Previous report suggests that the parasites in Thailand are selecting under mefloquine pressure and the proportion of higher MIC parasites is increasing[7].

Among 4 studied provinces, isolates from Ubon Ratchathani showed lower resistance to pyrimethamine, chloroquine and quinine, than those from other provinces. This result indicate that malaria parasites from different origins in Thailand have been "selecting" at different rates. The difference may reflect the different of parasite genetic and the regimen used in the areas. However, the actual reason is still unclear.

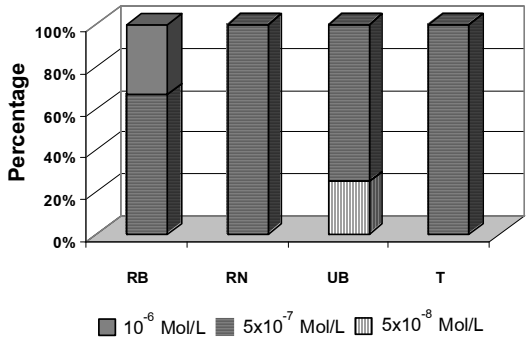


Figure 3 Drug susceptibility against chloroquine of all isolates from different parts of Thailand. Each colored bar represents a proportion of each MIC value in percentage. The MIC is reported as Molar concentration.

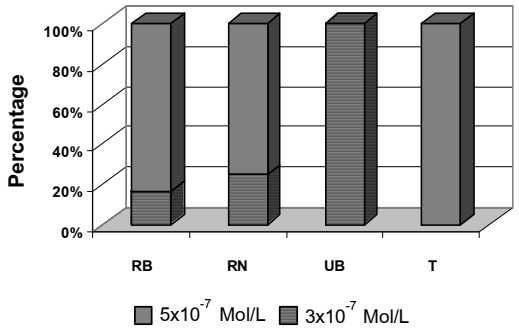


Figure 4 Drug susceptibility against quinine of all isolates from different parts of Thailand. Each colored bar represents a proportion of each MIC value in percentage. The MIC is reported as Molar concentration.

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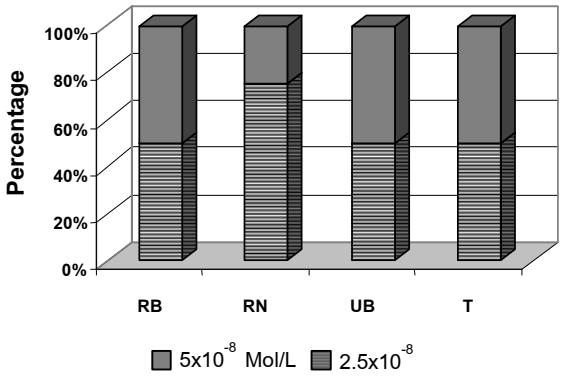


Figure 5 Drug susceptibility against amodiaquine of all isolates from different parts of Thailand. Each colored bar represents a proportion of each MIC value in percentage. The MIC is reported as Molar concentration.

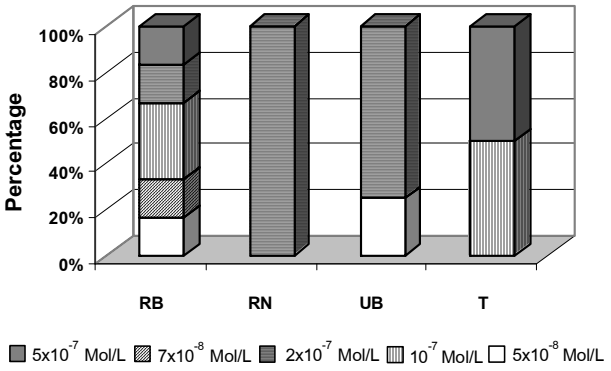


Figure 6 Drug susceptibility against mefloquine of all isolates from different parts of Thailand. Each colored bar represents a proportion of each MIC value in percentage. The MIC is reported as Molar concentration.

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ความไวต่อยาของเชื้อ *Plasmodium falciparum* ที่รวบรวมจากพื้นที่ต่าง ๆในประเทศไทยในระหว่างปี พ.ศ. 2543 – 2544

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บทคัดย่อ

เชื้อมาลาเรียจำนวน 18 ไอโซเลตถูกรวบรวมมาจาก (1) จังหวัดราชบุรี (2) จังหวัดตาก (3) จังหวัดระนอง (4) จังหวัดอุบลราชธานี ในจำนวนนี้มีเพียง 16 ไอโซเลตที่สามารถทำการเพาะเลี้ยงให้สามารถเจริญเติบโตได้ในสภาวะห้องปฏิบัติการ เชื้อที่เพาะเลี้ยงได้ถูกนำไปทดสอบกับยาต้านมาลาเรีย 5 ชนิด ได้แก่ ไพริเมธามีน คลอโรควิน ควินิน เมฟโฟลควิน และ อะโมไดอะควิน ช่วงของค่า MIC ที่ได้จากการทดลองในครั้งนี้มีค่าใกล้เคียงกับค่า MIC ที่มีรายงานไว้ก่อนหน้านี้ อย่างไรก็ตามเชื้อฟัลซิพารัมที่แยกได้จากจังหวัดอุบลราชธานีมีความไวต่อยาต้านมาลาเรียสูงกว่าไอโซเลตที่รวบรวมได้จากจังหวัดอื่นๆ แสดงว่าเชื้อมาลาเรียจากชายแดนไทย-ลาวและชายแดนไทย-พม่าอาจมีความแตกต่างกันในด้านพื้นฐานทางพันธุกรรม หรืออาจถูกคัดเลือกด้วยยาในอัตราที่แตกต่างกัน

คำสำคัญ: ฟัลซิพารัม ความไวต่อยาต้านมาลาเรีย ไพริเมธามีน คลอโรควิน ควินิน เมโฟลควิน อะโมไดอะควิน

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