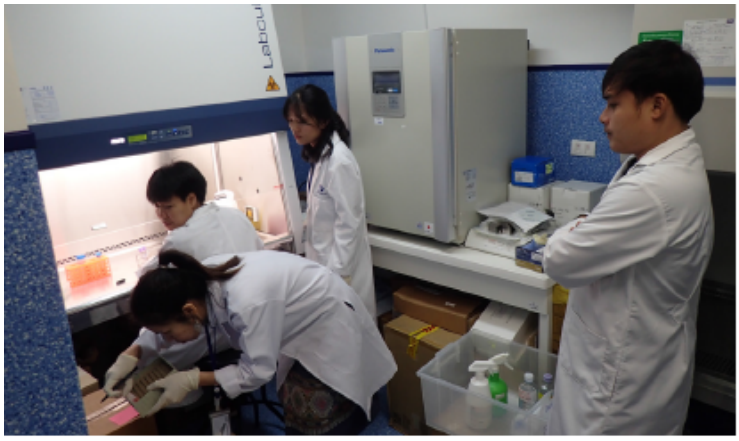
Antimalarial Drug Therapeutic Efficacy Study



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Background

Malaria caused by the protozoan parasite genus *Plasmodium* has been one of the medically important parasitic diseases in the Greater Mekong Sub-region (GMS) including Lao PDR. The morbidity and mortality of malaria have significantly decreased in the GMS over the last decade due to extensive efforts by the governments, partners and organizations, such as World Health Organization, Global Fund, Bill & Melinda

Gates Foundation [1]. The Lao Ministry of Health adopted an ambitious goal to eliminate *Plasmodium falciparum* malaria by 2025 and all forms of human malaria by 2030. Recently, artemisinin-based combination therapy (ACT) has been used for malaria treatment worldwide. In Lao PDR, Coartem (artemether-lumefantrine), one of the ACTs has been used for the treatment of *P. falciparum malaria* since 2004. However, clinical artemisinin resistance (or delayed parasite clearance) in *P. falciparum* was first reported in Pailin, the western part of Cambodia in 2009 [2] and gradually spread or emerged in the neighboring countries, which is a serious threat for malaria control and elimination in the GMS and globally.

Mutations of the *kelch13* gene *(k13)* in *P. falciparum* are associated with artemisinin resistance and currently used as a molecular marker for monitoring its resistance [3]. According to our previous survey for the artemisinin resistance in 2013, 20% (24/122) of *P. falciparum* isolates collected from malaria patients or villagers in the southern provinces possessed the resistant mutations in the *k13* gene [4]. Then, we had conducted a largescale artemisinin resistance survey using the *k13* marker in the five southern provinces since 2015 and in the northernmost province, Phongsaly, which is adjacent to China since 2017. Malaria patients' blood samples were collected on filter papers and DNA analysis was performed by PCR and DNA sequencing [5]. In the five southern provinces, 2,043 *P. falciparum* samples were analyzed by DNA sequencing. Percentages of the *k13* mutations were 55.7% (660/1,185) in 2015, 44.6% (179/401) in 2016 and 23.9% (109/457) in 2017. The predominant mutation was C580Y, which was also predominant in Cambodia, and followed by Y493H, R539T and P574L. The percentage of the mutations was higher in the two southernmost provinces, Champasak and Attapeu. On the other hand, in Phongsaly, all the *P. falciparum* samples (3/3) possessed the C580Y in 2017.

This study suggested the percentages of the *k13* mutations

seemed to be decreasing in Lao PDR. However, caution is needed because this tendency might be due to a relatively large number of *P. falciparum* samples from Savannakhet province with lower percentages of the mutation: 21.1% (35/166) in 2016 and 5.3% (14/266) in 2017. In addition, a mutation rate as high as 77.0% (47/61) was still observed in the southernmost province, Champasak in 2017. On the other hand, an antimalarial drug therapeutic efficacy study (TES) conducted by CMPE demonstrated that 10%-14% of cases were artemisinin resistance in 2013- 2017 [1]. This TES result with the prevalence of the *k13* mutations suggested that the efficacy of Coartem is slightly decreasing and the partner drug, lumefantrine is the main actor to clear malaria parasites in the patients. However, no molecular marker is identified for lumefantrine so far.

Objective

The objective of this study is to collect blood samples (living *P. falciparum*) from malaria patients in the field for laboratory analysis to identify and map resistant markers in the artemisinin partner drug, lumefantrine that is currently used in Lao PDR and many African countries for the first line treatment of malaria. A role of the Lao-Japan Parasitology Lab in IPL in this study is to preserve the sample of *P. falciparum* in liquid nitrogen in a collaboration with CMPE, the Ministry of Health and the local healthcare facilities in the study sites. The laboratory analysis will be conducted at Institut Pasteur du Cambodia (IPC).

Methodology

The study sites of this project are at public hospitals (provincial hospitals and district hospitals) in malaria endemic areas in Savannakhet, Salavan and Champasak provinces. Live *P. falciparum* samples are collected from malaria patients who participated in this study in Savannakhet, Salavan and Champasak provinces. The collected samples (approximately 2mL

of the fresh blood sample in EDTA tube) are sent by public bus in a thermos bottle with ice water to IPL and red blood cells are preserved in liquid nitrogen with a freezing solution. The live P. falciparum samples will be sent to IPC with dry shippers (special liquid nitrogen tanks) at the end of the project by air or land. Ethical clearance for this study was obtained by CMPE.

Results and future plan

A total of 74 samples (69 samples: Savannakhet; 5 samples: Salavan) was collected in this study and the red blood cells were stored in the liquid nitrogen tanks (Table 3). Serum samples and the remaining red blood cells were preserved in a freezer (-30oC) and filter paper, respectively. Currently, it is impossible to send the frozenlive *P. falciparum* samples to IPC because no airline or courier company that accepts frozen sample is available between Lao PDR and Cambodia due to the pandemic of COVID-19. The land border is also currently closed.

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References

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Driving to the study village