Overseas Practice on（Field Epidemiology・Collaborative Research） 2016/08/05

report form（For Student） (Year/Month/Day)

Name	NAN AYE THIDA OO
Laboratory	Division of Bioresources
Year (Grade)	Second year Ph.D student
Place of practice	Department of Medical Research
Period of practice	(160603 to 160723)
Purpose	To collect sputum samples for molecular detection of drug- resistant *M. tuberculosis*

Summary of activities（about 800 words, provide photos, tables and figures that clearly show the activities during the period）

Summary of activities for Field Epidemiology in Myanmar

Background and purpose: Tuberculosis (TB) caused by *M. tuberculosis* (MTB) still remains as one of the leading causes of morbidity and mortality in the world. Pyrazinamide (PZA) is first line anti-tuberculosis drug and activated by pyrazinamidase encoded by *pncA*. The previous study revealed that (41.1%) of MDR-TB had *pncA* mutations and 69 different varieties of mutation was dispersed on *pncA*. It seems that we need to clarify the possible correlation of each specific mutation to the emergence of PZA resistance. Therefore, the title of the study is phenotypic and genotypic analysis of pyrazinamide resistant MTB clinical isolates from Myanmar and the purpose is strengthening of sputum collection from Myanmar to do further molecular experiments of drug-resistant tuberculosis (DR-TB).

The objectives of the study were as follows:
-To detect phenotypic resistance to PZA on isolated MTB by Mycobacteria Growth Indication Test (MGIT)
-To evaluate the pattern of gene mutations conferring PZA resistance by DNA sequencing
-To elucidate the possible role of *pncA* mutations and bioactivity of PZase enzyme
-To find out the proportion of PZA resistance among new and retreatment pulmonary TB patients
In order to catch the purpose, research activities were performed in Department of Medical Research (DMR) especially Advanced Molecular Research Center (AMRC) and Bacteriology Research Division (B.RD), National Tuberculosis Reference Laboratory (NTRL) and State National TB program, Mawlamyaing (NTP-MLM).

Research Activities:

Research activities to get approval for sputum specimen's collections
The activities to get sputum samples were as followed and proceeded step by step.

- Submitted research and ethical proposal to the Ethical Review Committee of DMR, Ministry of Health and Sports (one month before leaving from Japan)
- Prepared presentation about research project
- Sited ethical defence presentation (on June 13th)
- Waited a couple of weeks to get the ethical approval (got ethical approval on June 22nd)

Research activities at AMRC, DMR

- Attended the BSL2+ laboratory training course (June 7-12)
- Did PZA and second-line drugs susceptibility test (DST) by MGIT
- Performed pyrazinamidase assay and sequencing of drug resistance-conferring mutation on *pncA, rpoB, katG, gyrA, gyrB, embB, rrs and eis*

Research activities at B.RD, DMR

- Isolation, identification and drug-susceptibility pattern of bacterial pathogens from clinical specimens
- Detection of carbapenemase producing *Enterobacteriaceae* by Vertek2
- Molecular characterization of drug resistance and virulence genes on the public concern pathogens (virulence genes of *Streptococcus pneumonia*, toxin- producing genes of *V.cholerae* and *Salmonella* spp)

Research activities at NTRL (Yangon)

- Primary isolation of MTB by L.J medium
- First and second-line DST by solid culture of L.J medium and liquid culture of MGIT
- Detection of rifampicin-resistant MTB by GeneX-pert/RIF assay

Research activities at Study area (NTP-MLM)

First trip to NTP-MLM (June 16 to 18)

- Made discussion with state TB officer about our research plan
- Asked agreement and opinions concerning about sputum collection on new and retreatment cases of TB
- Did detail discussion about the facts to be asked to the patients and how to transport specimens according to the Biosafety Guideline for Transporting of Infectious Materials
- Observed the services of NTP-MLM

Second trip to NTP-MLM (July 6 to 9)

- Started to make sputum collection at study area
- Observed and performed sputum processing and smear microscopy by Fluorescence Microscopy and DR-TB detection by GeneX-pert/RIF
- Helped in other activities of NTP-MLM such as giving of anti-TB drug (both DOTs and DOTs plus regimes) to the coming patients and collection of sputum for patients who enrolled as Patient Monitoring Drug Resistant Therapy

Research activities for collected sputum samples from study area (NTP-MLM)

Processing of sputum was performed by NaOH-NaLC method (Liquefaction / decontamination)

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Sediment

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Culture on L.J medium (total 52 specimen were finished up to this step)

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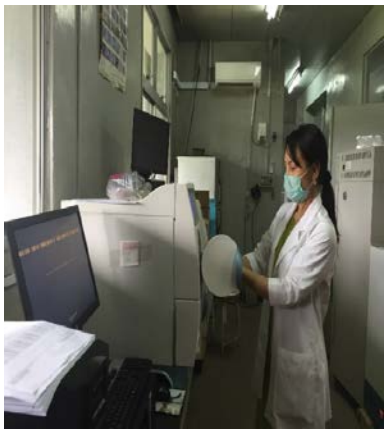
Isolation and identification of MTB, first-line anti-TB DST by solid culture, pyrazinamide susceptibility test by MGIT, PZase activity assay (will be carried out by our collaborators at DMR)

Advantages of Field Epidemiology Study

I have observed and performed research activities of the host institution's current works similar to my research plan. I got many advantages from this field study such as completion of BSL2+ training course, successful completion of culturing of TB from the collected samples, getting of hand on training of anit-TB DST by solid and liquid culture, safety extraction of DNA from live MTB and getting knowledge about target mission and challenges in controlling of TB and the emergence of DR-TB in Myanmar.

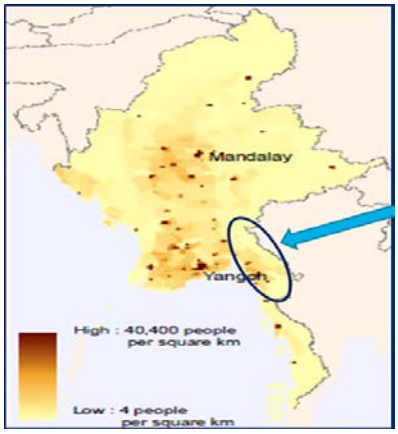
Conclusion

In this field epidemiology, we could start the new proposal for sputum collections from study area of interest and it could help in the further research works of pyrazinamide-resistant MTB from Myanmar isolates. Because of the slower growth nature of MTB, all research activities could not completely carry out during our limited stay. In addition, the time to get ethical approval for sputum collection takes two months and the starting time to collect samples was later than the expected date. Our collaborators are going to continue the research works and send DNA samples to us and we need to finish molecular characterization of pyrazinamide-resistant MTB from Myanmar isolates. The findings from this study could contribute to the development of rapid and accurate PZA susceptibility assay which can help in the control of TB in Myanmar.

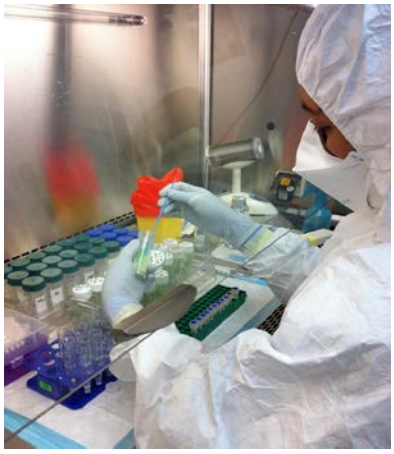


Perform lab work (Vertex2)

Collection of specimens



Field study area

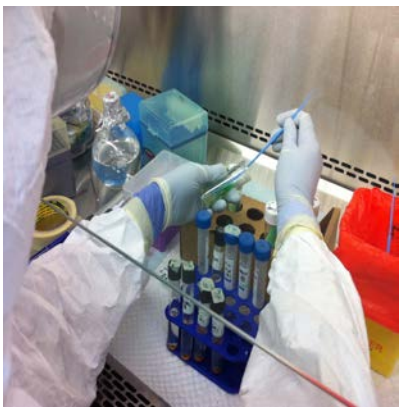


Culturing of *M. tuberculosis*

Attending group discussion

Field trip to NTP-MLM

NTRL (Yangon)



Performing PZase activity test

Host institution (DMR)

（Field Epidemiology・Collaborative Research）Evaluation by supervisor

Institution・Official title・Name	印
Describe overall evaluation on the applicant’s activity in overseas practice.	

※1 Send the electronic file to the Leading School section, International Affairs Office, also submit the original print out with seal of supervisor to the Leading School section, International Affairs Office.

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