e-ASIA Joint Research Program
Final Report

1. Project title：Whole-genome sequencing of drug-resistant *Mycobacterium tuberculosis* strains for diagnostics and outbreak detection

2. Joint Research period：07/01/2015 ～ 06/30/2018

3. Research Team：

■ **New Zealand team**（up to 6 people including the Principal Investigator）
Funding period: 07, 01, 2015 - 06,30,2018
Total Funded Amount (in Local Currency): NZD 450,000

	Name	Position	Affiliation	Role in the project
PI	Prof Gregory Cook	Professor	Department of Microbiology and Immunology Otago University	PI
Collaborator	Dr Htin Lin Aung	Sir Charles Hercus Research Fellow	Department of Microbiology and Immunology Otago University	Investigator, ECR
Collaborator	Prof John Crump	Professor	Center for International Health, Otago University	Investigator
Collaborator	Prof Philip Hill	Professor	Center for International Health, Otago University	Investigator
Collaborator	Prof Peter Lockhart	Professor	Massey University	Investigator
Collaborator	Dr Sally Roberts	Director	New Zealand Tuberculosis Reference Laboratory	Investigator
Total number of participants including students: 6				

■ **Myanmar team**（up to 6 people including the Principal Investigator）
Funding period: 07, 01, 2015 – 06,30,2018
Total Funded Amount (in Local Currency): In Kind

	Name	Position	Affiliation	Role in the project
PI	Prof Wah Win Htike	Professor	Department of Microbiology, University of Medicine 1	PI
Collaborator	Prof Kyi Kyi Thinn	Professor	Department of Microbiology, University of Medicine 1	Co-PI
Collaborator	Dr Thanda Tun	Consultant Microbiologist	Department of Microbiology, University of Medicine 1	Investigator
Collaborator	Dr Si Thu Aung	Programme Manager	National TB Programme	Investigator
Collaborator	Dr Wint Wint Nyunt	Consultant Microbiologist	National TB Programme	Investigator
Collaborator	Dr Thanda Lwin	Deputy Director General	Department of Public Health	Investigator
Total number of participants including students: 6				

■ **Indonesia team**（up to 6 people including the Principal Investigator）
Funding period: 07, 01, 2015 – 06,30, 2018
Total Funded Amount (in Local Currency): In Kind

	Name	Position	Affiliation	Role in the project
PI	Dr. Bachti Alisjahbana	Prof	TB-HIV research Center, Medical Faculty, Universitas Padjadjaran / Hasan Sadikin Hospital.	PI
Collaborator	Dr. Andriani Anggraini		Bandung Referral Laboratory	Investigator
Collaborator	Dr Lidya Chaidir	Head	TB Lab and Molecular Microbiology	Investigator
Collaborator				
Total number of participants including students: 3				

4. Summary of the joint research

Tuberculosis (TB) is a curable disease caused mainly by the bacterium Mycobacterium tuberculosis, and yet paradoxically it claims over 1.7 million lives annually. Of growing concern is the prevalence of multidrug-resistant TB (MDR-TB) and extensively drug-resistant (XDR-TB), being the main cause of deaths related to antimicrobial resistance (AMR). The World Health Organization (WHO) recognises that rapid detection of drug-resistant strains of M. tuberculosis is required to control the spread and transmission of MDR-TB and XDR-TB strains and to prevent the death of patients awaiting diagnosis. Conventional laboratory procedures for drug susceptibility testing (DST) take several weeks. To overcome this limitation, the Xpert MTB/RIF diagnostic test was developed. Xpert MTB/RIF is an automated diagnostic test that can identify M. tuberculosis DNA and resistance to rifampicin. Rifampicin resistance is considered a "surrogate marker" for detecting MDR- TB because more than 90% of all rifampicin clinical isolates are associated with isoniazid resistance, hence, MDR-TB. Unfortunately, this only interrogates the most frequent resistance mutations for rifampicin, resulting in excellent specificity but poor sensitivity for other drugs used to treat drug-resistant TB patients. Whole-genome sequencing (WGS) has recently been shown to overcome the shortcomings of current approaches to diagnosis in low burden settings.

In this study, we used WGS coupled with phenotypic DST to investigate the (1) drug resistance pattern, (2) genetic basis of drug resistance in and between countries, and (3) feasibility of using WGS for the management of TB and evaluate the performance of the Xpert MTB/RIF diagnostic test. We sequenced 300 and 150 Xpert RIF-resistant M. tuberculosis isolates respectively from Myanmar and Indonesia, two high drug-resistant burden countries in the South East Asia Region.

Our study revealed the high prevalence of resistance to second-line drugs among the MDR-strains that are circulating in Myanmar. Four percent of recruited MDR-TB cases (Xpert RIF-resistant cases) are found to be XDR-TB cases in both countries and 18% and 8% of MDR-TB cases represent pre-XDR cases in Myanmar and Indonesia respectively. We recommend that new classes of TB drugs are required to construct appropriate regimens.

We found that drug-resistant strains circulating in Myanmar harbor drug-resistance conferring mutations, which can be detected by the commercially available rapid point of care assays such as the GenoTypeTBDRsl. We

recommend that this be used as a rule-in test (a triage system) for pre-XDR and XDR cases, thereby using WGS to guide the treatment of these cases for cost-effectiveness. We used this approach to guide the treatment of two XDR-TB cases in Myanmar with new TB drugs as proof of concept. We also suggest a larger study using WGS for surveillance purposes at a national level, based on evidence in this study that there were direct transmissions of MDR-TB cases in the communities in Myanmar. In Indonesia, we found that (15% of isolates) discrepancies between Xpert RIF resistance test results and phenotypic DST; Further validation with targeted sequencing highlighted the potential to improve laboratory quality control system.

Through this study, we demonstrated that WGS can be used to (1) identify the drug-resistance pattern, (2) uncover the genetic basis of drug resistance, and (3) unravel transmission dynamics in high burden TB settings. The use of WGS can be expanded to other global infectious diseases including malaria and HIV. Therefore, we recommend this state-of-the-art WGS approach be used to provide evidence for effective policy intervention for targeted control measures, thereby facilitating the tackling of the significant AMR burden that the world is currently facing.

5. Outputs and Anticipated Outcomes of Joint Research

5-1 Scientific achievements and implemented activities of the joint research

We found that drug-resistant strains circulating in Myanmar harbor drug-resistance conferring mutations, which can be detected by the commercially available rapid point of care assays such as the GenoTypeTBDRsl. We recommend that this be used as a rule-in test (a triage system) for pre-XDR and XDR cases, thereby using WGS to guide the treatment of these cases for cost-effectiveness. We used this approach to guide the treatment of two XDR-TB cases in Myanmar with new TB drugs as proof of concept. We also suggest a larger study using WGS for surveillance purposes at a national level, based on evidence in this study that there were direct transmissions of MDR-TB cases in the communities in Myanmar. In Indonesia, we found that (15% of isolates) discrepancies between Xpert RIF resistance test results and phenotypic DST; Further validation with targeted sequencing highlighted the potential to improve laboratory quality control system.

In addition, this research has resulted in a number of scientific publications and been presented at scientific and non-scientific conferences to ensure the widest dissemination of the results to the public and research community.

5-2 Synergistic effects of the international joint research

This project combined international research expertise in TB at University of Otago, University of Medicine (1), and Universitas Padjadjaran, with the reference laboratories in the two high TB burden countries (Myanmar and Indonesia), along with external collaboration with world-leading researchers at the University of Cambridge, UK. This project addressed one of the greatest threats to global TB control, drug resistance, through the application of a state of the art technology in populations that are in most need. This project built completely new linkage between reference facilities and academic institutions in Indonesia and Myanmar involved in tackling the TB epidemic. The external link with the University of Cambridge enabled a link to a developing global consortium.

5-3 Broader impacts including contribution to society

Through this project, we have worked closely with the TB policymakers such as the National Tuberculosis Programme, Ministry of Health and Sports, Myanmar. Employing our experience in combatting TB in Myanmar, we are in collaboration with the New Zealand Tuberculosis Reference Laboratory to incorporate next-generation whole-genome sequencing (WGS) into the routine diagnosis of TB in New Zealand. This initiative is strongly in line with the WHO's call for strong political commitment at high level and multisectoral action using innovative technologies to expedite progress to combat TB. We will also provide evidence from this study to the TB healthcare policymakers in Indonesia. In addition, this research has resulted in a number of scientific publications and been presented at scientific and non-scientific conferences to ensure the widest dissemination of the results to the public and research community.

5-4 Development and sustainability of the cooperation

This research project has contributed to developing the health research workforce in New Zealand, as well as assisting in capacity building in New Zealand and developing countries. This research has allowed Dr Aung to become a significant leader in the use of WGS technologies to tackle infectious diseases. Dr Aung was awarded a prestigious Sir Charles Hercus Fellowship from HRC. The National Tuberculosis Programme in Myanmar is now evaluating the feasibility of incorporating WGS in the management of tuberculosis and drug-resistant tuberculosis in Myanmar. Building further on the successful foundations laid by this project, we aim to expand our research in collaboration

with Australia into other high TB burden countries in South East Asia such as Cambodia and the Philippines.

6. Future Goals and Plan of Activities after the project period

Building further on the successful foundations laid by this project, we aim to expand our research in collaboration with Australia into other high TB burden countries in South East Asia such as Cambodia and the Philippines.

7. Scientific Achievements and Implemented Activities (Publication, Research Exchange, Workshop, etc.)
For this item, please fill in the attached Excel file.

8. Recommendations and Comments to the Program

The e-ASIA Program represents a fantastic opportunity to assist in capacity building in developing countries and strengthening the international relationships.

9. Others (agenda of workshop, photos of research teams, meetings, and etc.)

Lists of Achievements and Implemented Activities

1. Original Publication of Articles etc.

1. 1 Original Publications (Articles co-authored among Research Teams)

All Authors' Names, Title, Journal Name, Volume, Edition, Page, Year of Publication	DOI Code	Publication Status	Remarks (e.g. publication in top level journals etc.)
Aung HL, Tun T, Moradigaravand D, Köser CU, Nyunt WW, Aung ST, Lwin T, Thinn KK, Crump JA, Parkhill J, Peacock SJ, Cook GM, Hill PC (2016) Whole-genome sequencing of multidrug-resistant Mycobacterium tuberculosis isolates from Myanmar. Journal of Global Antimicrobial Resistance 6: 113-117	10.1016/j.jgar.2016.04.008	published	
Tun T, Nyunt WW, Latt KZ, Samaranayaka A, Crump JA, Thinn KK, Cook GM, **Aung HL** (2016) Drug-resistant tuberculosis among previously treated patients in Yangon, Myanmar. International Journal of Mycobacteriology 5: 366-367	10.1016/j.ijmyco.2016.06.004	published	
Tun T, Aye KS, Nyunt WW, Crump JA, Nakajima C, Suzuki Y, Thinn KK, Cook GM, **Aung HL** (2016) Genotypic diversity of *Mycobacterium tuberculosis* strains in Myanmar. Infectious Diseases 49(3):237-239	10.1080/23744235.2016.1231419	published	
Aung HL, Tun T, Permina E, Nyunt WW, Aung ST, Thinn KK, Crump JA, Cook GM (2016) Draft genome sequences of the drug-resistant *Mycobacterium tuberculosis* isolates from Myanmar. Genome Announcements 4: (5).	10.1128/genomeA.00850	published	
Nyunt WW, Aung ST, Lwin T, Cook GM, **Aung HL** (2017) First- and second-line anti-tuberculosis drug resistance patterns among previous treatment failure patients in Myanmar. Journal of Global Antimicrobial Resistance 9:34-35.	10.1016/j.jgar.2017.02.007	published	
Nyunt WW, Aung ST, Cook GM, Aung HL (2017) Evaluation of the MTBDR*sl* test for detection of second-line resistance in drug-resistant *Mycobacterium tuberculosis* strains in Myanmar. Infectious Disease 49:(11-12) 865-866	10.1080/23744235.2017.1341056	published	
Aung HL, Tun T, Nyunt WW, Fong Y, Crump JA, Thinn KK, Aung ST, Cook GM. (2017) Association between mutations in anti-tuberculosis drug resistance-conferring genes and treatment outcomes in Myanmar. Infectious Diseases	10.1080/23744235.2017.1404632	published	
Aung HL, Nyunt WW, Fong Y, Cook GM, Aung ST., (2017) First two extensively drug-resistant tuberculosis cases from Myanmar treated with bedaquiline. Clinical Infectious Diseases 65(3):531-532	10.1093/cid/cix365	published	
8	Total		

1. 2 Original Publications (Articles by Single Team only)

All Authors' Names, Title, Journal Name, Volume, Edition, Page, Year of Publication	DOI Code	Publication Status	Remarks (e.g. publication in top level journals etc.)	Country name of the team
Mulholland CV, Ruthe A, Cursons RT, Durrant R, Karalus N, Coley K, Bower J, Permina E, Coleman MJ, Roberts SA, Arcus VL, Cook GM, **Aung HL** (2017) Rapid Diagnosis of the *Mycobacterium tuberculosis* Rangipo strain responsible for the largest recurring TB cluster in New Zealand. Diagnostic Microbiology and Infectious Disease 88:138-140	10.1016/j.diagmicrobio.2017.03.012	published		New Zealand
Mulholland CV, Thorpe D, Cursons RT, Karalus N, Fong Y, Arcus VL, Cook GM, Aung HL (2018) Evaluation of the Rapid Molecular Diagnostic Test for the New Zealand *Mycobacterium tuberculosis* Rangipo Strain in a Clinical Setting. New Zealand Medical Journal 131(1478):70-72		published		New Zealand

2	Total

Lists of Achievements and Implemented Activities

2. presentations at Academic Conferences etc. (Seminars, Workshops, Symposia)

2. 1 Conference Presentations (Joint Presentations among Research Teams)

Date	Type of Presentation	Speaker, "Title", Conference Name, Location, etc.
March 5, 2017	Guest/Invited Speaker	Htin Lin Aung, The implication of whole-genome sequencing in the control of tuberculosis, 24th Myanmar Military Medical Conference, Yangon, Myanmar
December 13 2017	Oral Presentation	Htin Lin Aung, Combatting Tuberculosis at International Frontlines , 3rd One Health Aotearoa Conference, Wellington, New Zealand
February 22, 2018	Guest/Invited Speaker	Htin Lin Aung, Next- and third generation sequencing as a molecular weapon to combat TB on the frontline in Myanmar, 25th Myanmar Military Medical Conference, Yangon, Myanmar

3 Total

2. 2 Conference Presentations (by Single Team)

Date	Type of Presentation	Speaker, "Title", Conference Name, Location etc.	Country name of the team
January 5, 2016	Oral Presentation	Thanda Tun, Multidrug-Resistant Mycobacterium tuberculosis strains in Myanmar patients, 44th Myanmar Health Resarch Congress, Yangon, Myanmar	Myanmar
November 11, 2015	Guest/Invited Speaker	Htin Lin Aung, Whole-genome sequencing for the management of drug-resistant tuberculosis, 8th Otago International Health Research Network Annual Conference, Dunedin New Zealand	New Zealand
September 7, 2017	Guest/Invited Speaker	Htin Lin Aung, The implications of Whole-genome sequencing for the management of drug-resistant tuberculosis , National Symposium on Infectious Diseases: Key Opportunities and Emerging Therapies, Queenstown, New Zealand	New Zealand

3 Total

Lists of Achievements and Implemented Activities

3. Workshops, Seminars, Symposia and Other Events (Organized by the Project)

Event duration	Name of Organizer	Title of the Event	Location (Country, City, Venue)	Number of Participants (Including Team Members)	Overview
February 9-13 2016	Dr Htin Lin Aung, Professor Sharon Peacock	WGS workshop	Yangon, Myanmar	15	

1 Total

Lists of Achievements and Implemented Activities

4. Record of Research Exchanges

Date of Departure	Date of Return	Last Name & First Name	Country of Affiliation	Affiliation	Position	Exchange Destination (Country, City, Research Organization etc)	Description of Exchange Content/Purpose	Duration of Exchange (autocompleted)
October 14, 2015	October 20, 2015	Aung, Htin Lin	New Zealand	University of Otago	Dr	University of Medicine 1 and National TB Programme, Myanmar		7
October 21, 2015	October 26, 2015	Aung, Htin Lin	New Zealand	University of Otago	Dr	Universitas Padjadjaran / Hasan Sadikin Hospital Bangdaung Referral Laboratory, Indonesia		7
Octobber 14, 2015	Octobber 20, 2015	Hill, Philiip	New Zealand	University of Otago	Professor	University of Medicine 1 and National TB Programme, Myanmar		7
October 21, 2015	October 26, 2015	HIll, Philiip	New Zealand	University of Otago	Professor	Universitas Padjadjaran / Hasan Sadikin Hospital Bangdaung Referral Laboratory, Indonesia		7
February 9, 2016	February 13, 2016	Aung, Htin Lin	New Zealand	University of Otago	Dr	University of Medicine 1 and National TB Programme, Myanmar		4
July 9 2016	July 15 2016	Aung, Htin Lin	New Zealand	University of Otago	Dr	University of Medicine 1 and National TB Programme, Myanmar		6
March 6, 2017	March 10 2017	Cook, Gregory	New Zealand	University of Otago	Professor	University of Medicine 1 and National TB Programme, Myanmar		4
July 15, 2017	July 20 2017	Hill, Philiip	New Zealand	University of Otago	Professor	Universitas Padjadjaran / Hasan Sadikin Hospital Bangdaung Referral Laboratory, Indonesia		6
February 19 2018	February 23, 2018	Cook, Gregory	New Zealand	University of Otago	Professor	University of Medicine 1 and National TB Programme, Myanmar		5
February 19 2018	February 23, 2018	Aung, Htin Lin	New Zealand	University of Otago	Dr	University of Medicine 1 and National TB Programme, Myanmar		5
					Total (Person)	9	Total (Persond-day)	51

Lists of Achievements and Implemented Activities

5. Patent Applications

5. 1 Independent Applications by Single Team

Application Number	Name of Patent/Patent Name	Application Date	Patent Applicants (Fill in All Members)	Publication Number (leave blank if unpublished)	Inventor	Country of Application	Registration Number (leave blank if unregistered)	Country Name of the Team

0 Total (Number of Application)

0 Total (Number of Registration)

5. 2 Joint Applications

Application Number	Name of Patent/Patent Name	Application Date	Patent Applicants (Fill in All Members)	Publication Number (leave blank if unpublished)	Inventor	Country of Application	Registration Number (leave blank if unregistered)

0 Total (Number of Application)

0 Total (Number of Registration)

Lists of Achievements and Implemented Activities

6. Awards

Date of Award	Name of Award	Recipient	Remarks	Country Name of the Team
September 3, 2015	New Zealand – ASEAN award	Htin Lin Aung		New Zealand
January 9, 2016	Second Prize for Best Paper, 44th Myanmar Health Research Congress	Thanda Tun		Myanmar
July 6, 2017	University of Otago Resarch Group Award	Htin Lin Aung, Gregory Cook		New Zealand
November 2, 2017	Sir Charles Hercus Health Research Fellow	Htin Lin Aung		New Zealand
July 27, 2018	ZICO ASEAN 40 Under 40 Award	Htin Lin Aung		New Zealand
August 28, 2018	illumina Emerging Researcher Award	Htin Lin Aung		New Zealand
6	Total			