MOLECULAR TECHNOLOGY APPLICATIONS DIVISION

Deputy Director & Head	...	Dr. Yi Yi Kyaw MBBS, MMedSc(Microbiology)(UM1)
Research Scientist	...	Dr. Myat Htut Nyunt MBBS(UM2), MMedSc(Microbiology) (UM1), DAP&E(IMR)(Malaysia), PhD(Parasitology)(ROK)
	...	Daw Ohnmar Lwin BSc(Zoology)(YU), Dip in Korean(YUFL)
	...	Dr. Phyu Win Ei MBBS(UM2), MMedSc(Microbiology)(UM1)
Research Officer		Dr. Zayar Han MBBS(UM2)
	...	Dr. Hnin Ohnmar Soe MBBS, MMedSc (Pathology)(UMM)
	...	Dr. Cho Thanda Tun BSc(Hons;), MSc, M.Res, PhD(Physics)(YU)
	...	Daw Mi Mi Htwe BSc(Zoology), MSc(Zoology)(YU)
Medical Technician (1)	...	Daw Ni Ni Zaw BA (Myanmarsar)(DU)
Research Assistant (2)	...	U Saw Ba Win BSc(Physics)(DU), Diploma in Applied Physics, MSc(Engineering Physics)(YU)
	...	Daw Cho Cho BSc(Hons;)(Zoology)(YU)
Research Assistant (3)	...	Dr. Su Mon Win BSc(Engineering), MSc, M.Res (Biotechnology), Ph.D(Zoology)(YU)
	...	Daw Wai Myat Thwe BSc(Botany)(UDE)
	...	Daw Thin Thin Aye B.Sc(Chemistry)(YU)
Research Assistant (4)	...	U Phyo Wai Kyaw
	...	Daw Nilar Shwe
	...	Daw Win Win Khine
Laboratory Attendant		Daw Naw Zar Htoo BA(Geography)(WYU)
		Daw Khaing Khaing Wai AGTI(Mechantronic)
		Daw Shunn Lei Naing B.Sc(Hons;)(DU), MSc(Biochemistry)(YU)

The Molecular Technology Applications Division is involved in research on communicable diseases; mainly on tuberculosis, viral hepatitis and malaria. The main research areas include establishment of molecular techniques to detect asymptomatic/ silent infections, genotyping and detection of gene mutations conferring drug resistance as well as applications of these methods for diagnosis, characterization and molecular epidemiology. The division is also responsible for maintenance of laboratory equipment and Biosafety level 2 plus laboratory.

RESEARCH PROJECTS

1. COMMUNICABLE DISEASES

1.1 TUBERCULOSIS

1.1.1 Detection of extensively drug-resistant tuberculosis (XDR-TB) among clinical *Mycobacterium tuberculosis* isolates in Myanmar (2016)

Multidrug resistant TB (MDR-TB) does not respond to the standard six month regimen with first line anti-TB drugs and takes at least two years to treat with second line anti-TB drugs which are less effective and more toxic. Extensively drug resistant tuberculosis (XDR-TB) is defined as MDR-TB strains that are also resistant to treatment with any fluoroquinolone and any of the injectable second-line anti-TB drugs. Pre–XDR TB is defined as TB with resistance to isoniazid and rifampin and either a fluoroquinolone or second-line injectable agent but not both. Pre XDR condition in a TB patient is important because it will turn into resistance easily and the case will become XDR TB. The objectives of this study are to detect XDR-TB cases among MDR TB patients attending Yangon and Mandalay TB Centres and to determine gene mutation pattern conferring fluoroquinolones and aminoglycoside resistance. Phenotypic anti-TB drug susceptibility testing was carried out by using solid culture M-KIT plates. Sanger sequencing of target genes for fluoroquinolones (*gyrA*, *gyrB*), and aminoglycosides (*rrs*, *eis*) were performed using ABI3500 sequencer. During 2016, 31 MDR-TB isolates from 20 males and 11 females with mean age of 35.8$\pm$10.9 years were analyzed. Among them, 16.1% (5/31) are XDR cases and approximately 26% (8/31) pre-XDR, of whom, 7 were fluoroquinolone resistant pre-XDR (5 were resistant to ofloxacin, levofloxacin and moxifloxacin; 2 were resistant to ofloxacin and levofloxacin)and one was all injectable (amikacin, kanamycin and capreomycin) resistant pre-XDR. Four different types of mutations were detected in g*yr A*gene (D94G, D94Y, S95T, D94N)and one mutation was detected in *rrs*gene. No mutation was found in *gyrB* or *eis*gene. In *gyrA* gene, mutation in codon 94 (D94G) was the most common mutation in as it was found in 4 of 9 phenotypically ofloxacin resistant isolates. In *rrs*gene, mutation in nucleotide 1401 (CAC-CGC, His-Arg) was found in 4 phenotypically all injectable resistant isolates. The mutation pattern found in the present study was similar to the common mutation pattern reported by the other countries. The present findings provide the information on the occurrence of XDR-TB and prevalent mutation pattern of fluoroquinolone and aminoglycoside resistant *M. tuberculosis* strains in Myanmar. Prevalent mutation pattern of the drug target genes can be useful for development as well as improvement of rapid diagnosis tool for drug resistance.

1.1.2 Genetic diversity and drug susceptibility of *Mycobacterium tuberculosis* strains in Myanmar: Comparison between new and retreated cases

[Molecular typing and detection of drug resistant mutation in MDR-TB strains in Myanmar (2016)]

Genotyping of *Mycobacterium tuberculosis* (MTB) strains reveals the tracts of infection in disease epidemiology and it can correlate with the disease's clinical nature and outcomes after treatment. Retreatment cases include patients with relapse, treatment after default and treatment after failure and are accepted as high risk group for development of multidrug resistant (MDR) TB. The present study was carried out to determine and compare the genetic diversity and drug susceptibility pattern of MTB strains isolated from new and retreatment pulmonary tuberculosis patients in Myanmar. A total of 105 clinical MTB strains

isolates from new (n=60) and retreatment (n=45) cases were studied in 2016. Drug susceptibility test was done on four first line drugs on solid media. Genotype detection was done by internationally standardized 24 loci MIRU-VNTR typing method as well as a PCR-based typing method to identify Beijing and Non-Beijing strains. MIRU-VNTR typing results were analyzed by the MIRU-VNTR*plus* web application. The drug susceptibility test result of 60 new patients included 12 MDR, 4 any drug resistance other than MDR and 44 susceptible strains and that of the 45 retreatment cases revealed 39 MDR and 6 any drug resistance other than MDR without a pan-susceptible strain. On genotyping, all tested 105 MTB isolates showed unique patterns and did not clustered. The combined results of two typing methods showed the strains distributed into four major lineages; Beijing (77/105, 73.33%), East-African-Indian (4/105, 3.80%), Uganda 1 (2/105, 1.90%), CAS/Delhi (1/105, 0.95%) and unknown non-Beijing strains (21/105, 20%) as overall. Eight samples showed both Beijing and non-Beijing characters and comprised as suspected co-infected cases. Among 60 new MTB strains, 37 (61.66%) were Beijing genotype (including 7 suspected co-infection) and 23 (21.90%) were non-Beijing strains (4 EAI, 1 CAS/Delhi and 18 unknown). Among 45 strains from retreatment cases, 40 (88.89%) were Beijing strains (including 1 suspected co-infection case) and 5 (11.11%) were non-Beijing strains. Retreatment cases were strongly associated with MDR (P = 0.0001) and Beijing family strains were significantly associated with occurrence of retreatment cases (P = 0.0018). The result data suggested Beijing strains of MTB may be more intended to infect than other strains or have a tendency to recur or develop into MDR and it should be suggested that the areas where Beijing genotype is prevalent should practice more intense follow up system even after full treatment.

1.1.3 Pyrazinamide resistance in rifampicin resistant *Mycobacterium tuberculosis* isolates in Myanmar

Pyrazinamide (PZA) is a key component of short-course anti-tuberculosis treatment regimen and also of second-line regimen for multidrug-resistant TB (MDR-TB) treatment. PZA susceptibility testing is rarely performed routinely because of technical difficulties. A recent population–based multi-country survey led by the World Health Organization indicated the burden of PZA resistance among patients with rifampicin resistance and encouraged the PZA resistance surveillance in different settings. This study was carried out to detect proportion of PZA resistance and *pncA* mutations responsible for PZA resistance in *Mycobacterium tuberculosis* (MTB) strains from Myanmar. During 2015-2016, rifampicin resistant (RR) MTB clinical isolates were collected from Yangon and Mandalay TB Centres. Phenotypic PZA resistance was detected by liquid culture based Mycobacterial Growth Indicator Tube (MGIT) method and mutations in the *pncA*gene were detected by DNA sequencing using ABI 3130 genetic analyzer. Among 40 RR-MTB isolates, PZA resistance was found in 26 (65.0%) by MGIT test. In PZA resistant phenotypes, 31 different types of mutations were distributed on the *pncA*gene and 10 types of which were found to be novel mutations. Common mutations were found at the following regions of each of two strains: Lys96, Phe81, Thr135, Gly17 and Thr61 (5% each).The present study showed the proportion of PZA resistance and strong correlation between *pnc*A mutations and phenotypic PZA resistance (Cohen's Kappa Index =0.94). PZA resistance information can contribute to the National TB Program and supported the fact that routine PZA susceptibility testing is needed to be incorporated to current monitoring regimen and National drug resistance surveys.

1.2 VIRAL HEPATITIS

1.2.1 Hepatitis B infection

1.2.1.1 Detection of hepatitis B virus X and core promoter mutations and gene expression study on Myanmar patients with HBV induced hepatocellular carcinoma (2016)

Chronic hepatitis B virus (HBV) infection is the most common cause of hepatocellular carcinoma (HCC) in Asia. It has been suggested that viral factors including HBV-DNA levels, genotypes, and genomic mutations, and host properties including age, sex, race, and immune status, as well as unhealthy life style, might contribute to the progression of liver diseases leading to hepatocellular carcinoma. Among the viral factors, HBV X gene and overlapping area of core promoter region are critical for viral transcription and is related with the development of HCC. This study was conducted to find out the viral mutation and relation to development of HCC. The present study involved HBV surface antigen (HBsAg) positive cases with HCC (n =10, 8 male and 2 female, mean age 55.6 ± 11.6-year -old) admitted to the 500 bedded Specialist Hospital and chronic hepatitis B patients (all are female, age 33.8 ±13.4-year old) those who attending at the Hepatitis Clinic from Department of Medical Research. Viral DNA detection was carried out with core ORF region and further sequenced to core promoter region (including enhancer II)which is the partial area of core gene by PCR-direct sequencing method using with ABI 3500 Genetic Analyzer. The resultant sequences were aligned and compared with wild type reference sequences using with Bioedit software. Ten HCC samples were also sent to sequencing company for whole genome sequencing to detect viral genotyping and other genes changes and amino acid changes. Among the Core promoter region of HBV virus, Basal Core Promoter (BCP) region, C1638T and T1753V mutations constituted independent risk factors for the advancement of liver diseases. The presence of C1638T mutation was seen on chronic hepatitis cases as well as liver cancer patients in our study. T1753V mutation was seen on 7 out of 10 HCC patients but not seen in chronic hepatitis B patients. In BCP region, double mutations, A1762T and G1764A mutations were detected in 8/10 (80%) and single mutation, A 1764 G 2/10 (20%) was seen on two hepatocellular carcinoma cases. In comparison with chronic hepatitis patients, none of cases were seen on double mutation or single mutation at BCP region. The presence of G1896A, T1771C and A1773G mutations were seen on most of HCC patients but single point mutation C1786 T, C1788A, T 1784 C mutation were seen on precore promoter region of chronic hepatitis patients. In this study, double mutation was significantly higher in cases with Hepatocellular carcinoma than chronic hepatitis patients.

1.2.1.2 Molecular characterization of viral hepatitis in Myanmar

The aim of the study is to investigate the molecular Characteristic of hepatitis viruses in Myanmar. A total 75 Hepatitis B samples those who attending the Hepatitis Clinic at year 2013 were collected and confirmed by Biomurex HBs Ag ELISA 3.0 for confirmation. Viral DNA detection was carried out on all hepatitis B blood samples and 30 samples were sequenced on PreS region of HBV genome. The most of the samples (19/30) were genotype D (63.3%) follow by genotype C (9/30) 30% and genotype A (1/30) 3.3% and genotype B (1/30) 3.33%.Partial Genome Direct Sequencing Method for hepatitis B was established at Department of Medical Research and it will be useful as basic data for Hepatitis Control Programme in Myanmar

1.2.2. HIV and Hepatitis B Co-infection

1.2.2.1 Prevalence of HBV markers in people living with HIV patients

Hepatitis infection is a leading cause of chronic hepatitis, liver cirrhosis and hepatocellular carcinoma worldwide. Due to the shared mode of transmission, co-infection with Hepatitis B Virus (HBV) and Human Immunodeficiency Virus (HIV) is not uncommon. With the objective to determine the hepatitis B surface antigen (HBsAg), hepatitis B core antibody (anti-HBc), hepatitis B e antibody (anti-HBe), hepatitis B e antigen (HBeAg) and hepatitis B surface antibody (anti-HBs) prevalence among HIV infected patients and identify the association between socio-demographic variables and the HBV infection markers, this research was conducted in Wai Bar Gi Specialist Hospital from July 2016 to October 2016. A total of 131 HIV-seropositive patients were enrolled. HBV markers were tested with Combo Test Kit. Out of 131 persons, male comprises 74 (56.5%) and 57 (43.5%) were females. Patients aged over 40 years were 83 (63.36%) and under 40 years were 48 (36.64%).The overall positive result for HBsAg was 19 (14.5%), Anti-HBs was 39 (29.77%), HBeAg was 3 (2.29%), anti-HBe was 6 (4.58%) and anti-HBc was 20 (15.27%) respectively. Furthermore, 85.5% of the patients were classified as never having been infected. The positivity of HBsAg in this research work (14.5%) is not high significantly than the National result (10 – 13%). It is recommended that HIV infected individuals with negative HBsAg should be immunized against HBV when they were seen first at Out Patient Departments of Government Hospitals, Private Hospitals and Private Clinics. By this way HBV infection transmissions and its sequelae can reduce to a certain extent.

1.3 MALARIA

1.3.1 Drug Resistant Malaria

1.3.1.1 Assessment of drug resistance molecular markers in asymptomatic malaria in Myanmar

One year prospective observation study was conducted in 1182 apparently healthy residents in MARC Zone I area in 2015. Malaria infection was assessed by active cases detection in every three months by rapid diagnostic test, microscopy and molecular analysis. Artemisinin resistance molecular markers such as K13 (kelch 13 propeller gene), *pfarps10* (*Plasmodium falciparum* apicoplast ribosomal protein S10), *pffd* (*P. falciparum* ferredoxin) and *pfmdr2*(*P. falciparum* multidrug resistance protein 2)in falciparum infection and antimalarial drug resistance markers in vivax infection such as *pvcrt-o*, *pvmdr1*, *pvdhps* and *pvdhfr* were analyzed. Asymptomatic infection was observed as 2.5% (30/1182), 0.7% (6/894), 0.4% (13/944) and 0.9% (8/889) in four times active detections respectively. Two out of four falciparum isolates showed K13 mutations (C580Y in one isolate and P574L in another one) and *pfarps10* mutation (V127M) in two, *pffd* mutation (D195Y) in three and *pfmdr2* (T484I) mutation in all four isolates were noted. Among 45 vivax isolates, high mutation rate was observed in known drug resistance markers such as *pvcrt-o* (79.5%, 35/44), *pvhdps* (100.0%, 43/43), *pvdhfr* (100.0%, 44/44) and *pvmdr1* (100.0%, 44/44) and mutants pattern was similar to that of symptomatic vivax infection in the same areas. Drug resistance in asymptomatic infection should not be neglected even in healthy residents in containment zone and may pose to challenges in elimination of malaria. Our evidence highlights the need of strategy with strong efforts to eliminate the multi-drug resistance malaria in asymptomatic infection.

1.3.2 Field Research on Malaria

1.3.2.1 Sero-epidemiology and Malaria Antibody Kinetic Profile in Tier I area of Myanmar Artemisinin Resistance Containment (MARC) Zone

Sero-epidemiology and sero-kinetic against the merozoites surface antigens of *P. falciparum* and *P. vivax* among the residents in Shwegyin Township was conducted using the sera collected in one year observational study in 2015. The top five candidates that showed highest antigenicity on acute or subacute infection were selected for recombinant protein expression by *E. coli* based system. Antibodies specific for the well-expressed recombinant blood stage merozoite surface antigens, PfMSP1-19 and PvMSP1-19, PvAMA1, and PvDBPII were screened in 1080 sera samples of 270 participants from four times collections by using protein microarray in duplicate manner. Seropositivity rates against the PfMSP1-19, PvMSP1-19, PvAMA1 and PvDBPII were 73/270 (27.0%), 85/270 (31.5%), 65/270 (24.1%) and 160/270 (59.3%), respectively. The PvDBPII showed high and stable seropositivity especially in older age group without evidence of past infection, reflecting long-lasting humoral immune response. Similarly, stable seropositivity rate of PvAMA1 was observed in acute, subacute and history samples, and all four time collected sera regardless of age and sex group as well as past infection history, indicating its limited usefulness as a serological marker. On the other hand, PfMSP1-19 and PvMSP1-19 showed high and stable antigenicity in acute and subacute samples but declining in one year history samples. No cross reactivity of PfMSP1-19 and PvMSP1-19 between the two species and higher seropositivity among the asymptomatic carriers were observed. Our evidence suggests that PfMSP1-19 and PvMSP1-19 are the leading candidate markers for serological analysis of malaria where both falciparum and vivax are common.

1.4 CHOLERA

1.4.1 Molecular characterization of *Vibrio cholerae* in Myanmar(2016)

Change in climate or weather *can affect the emergence and spread of cholera in human populations.* During May to October 2015, 331 bacteriologically confirmed cholera cases were reported in Mandalay Region, Myanmar, In the present study 67 *Vibrio cholerae* isolates of these cases were characterized by DNA sequencing, pulse field gel electrophoresis (PFGE) and multiple locus variable number tandem repeat analysis (MLVA). Forty two isolates were from male and 25 from female patient with the mean age of 26 year ranging from 8 month to 68 years. All the isolates carried cholera toxin genes (*ctxAB*), cholera toxin B subunit gene of the classical biotype ($ctxB^{cla}$), biotype-specific CTX prophage repressor gene of El Torbiotype ($rstR^{El}$) and the toxin-coregulated pilus A gene of Haitian type ($tcpA^{CIRS}$).The tested isolates were similar to the CIRS101, and CIRS101-like variants that had been found in Nigeria, Pakistan, Afghanistan, South Africa, Sri Lanka, Vietnam, India, Bangladesh, and Thailand. PFGE typing of *Not*I digests of the 67 isolates differentiated into 4 pulsotypes designated as Y12, Y15, Y16, and Y17.Y15 was the most present pulsotype, accounting for 92.5% (62/67) of the isolates. In addition, MLVA at five loci yielded 7 types, and 64 isolates (96%) exhibited either identical MLVA profile of previous reports or closely related profiles that differed only by 1 repeat number. These data suggested that a clonal *V. cholerae*O1 exhibiting identical PFGE and MLVA type spread in Mandalay during the rainy season, 2015. When comparing the molecular characteristics of 2015 Mandalay *V. cholera* strains with 22 *V. cholerae*O1 strains (12 from Mandalay and 10 from Yangon) isolated in 2014, all 2014 isolates were also toxigenic *V. cholerae* O1, biotype El Tor, serotype Ogawa, carrying genes $ctxB^{cla}$, $rstR^{El}$, and $tcpA^{CIRS}$. Four Yangon isolates exhibited the pulsotypes Y12 or Y15

and MLVA profile identical to the 2015 Mandalay isolates while 2014 isolates from Mandalay were comparably far related. Although the number of test isolates was limited, it appears that cholera in Mandalay 2015 were related to the incidences in Yangon 2014 rather than in Mandalay 2014. Pulsotypes Y12 and Y15 were not previously observed in Yangon, at least, during 2012 to 2013. Therefore, it is likely that rapid transmission of the Yangon clone to Mandalay occurred. *V. cholera* infections occur globally and Myanmar isolates from two remote areas were also linked to the wave 3 of the seventh cholera pandemic that might have spread from the Bay of Bengal.

2. ACADEMIC AND TECHNOLOGY DEVELOPMENT

2.1 Usefulness of dried blood spot samples for detection, quantification, and molecular characterization of HBV DNA in Myanmar

Hepatitis B virus (HBV) infection is regarded as a significant public health problem in Myanmar because of the ability of HBV to induce a chronic carrier state. Even though chronic hepatitis B carriers remain largely asymptomatic, many of these individuals subsequently develop cirrhosis and primary hepatocellular carcinomas. Dried blood spot (DBS) samples are a simple and inexpensive sampling method, particularly useful for blood collection in resource-poor settings with limited access to diagnostic facilities. Thirty DBS were collected on filter paper (Whatman FTA ^{TM} from GE Health Care Bio Sciences Coop, USA) as well as whole blood samples from same HBs Ag positive patients from Nay Pyi Taw, Central Myanmar and Tachileik, Eastern Shan States. The filter papers were transported to DMR with room temperature. For the whole blood samples, the sera were separated within four hours after blood collection and transported to DMR by cold chain. HBV DNA from filter papers can be extracted from filter paper and conventional PCR and real-time PCR can be carried out. Twenty samples are positive for HBV DNA and 100 percent agreement with blood samples from same patients. Seven (Four from Nay Pyi Taw and three from Tachileik) which were strong PCR positive samples can be genotyped using with partial genome sequencing of PreS region. Genotyping was carried out with NCBI HBV genotyping tool using with reference sequences. Five sequences showed Genotype C and two sequences was genotype D. This study highlighted that DBS is a suitable alternative sample transport method from hard to reach area of Myanmar to test for HBV genotyping and viral amount quantification. DBS sampling allows for HBVtesting and treatment follow-up for infected patients residing in areas of Myanmar difficult to access.

3. RESEARCH CAPACITY STRENGTHENING

3.1. Maintenance of Biosafety level 2 plus laboratory (BSL2+) and laboratory equipment

The following services were carried out for maintenance of BSL2+ laboratory and laboratory equipment.

- Regular checking to use type I water (molecular grade) from water purifier
- Regular checking and maintenance of Uninterruptable Power Supply (UPS) for current over load
- Regular checking of ice making machine and gas filling
- Checking and repair of frequency control unit of ceiling exhaust fan
- Changing of the prefilters, median filters and HEPA filters of BSL2+ laboratory and fixing and changing of frequency relay Condensing Unit (CDU)
- Servicing of air conditioners of AMRC and DNA sequencer room
- Reporting of AHU System check two weekly to Wosem Company
- Assist on repair of Programmatic Logic Control System

3.2. Maintenance Schedule

- Daily checking of all laboratories equipment, lighting and air conditions
- Checklist of AHU (Air handling Unit) and EFU (Exhaust Fan Unit) twice a week
- Checklist of all laboratory equipment twice a month
- Cleaning and changing of pre-filter twice a month, medium filter three monthly and HEPA filter once a year in BSL2+ laboratory

SERVICE PROVIDED

ACADEMIC

Sr.	Name	Course	Responsibility
1.	Dr. Yi Yi Kyaw	1st year Diploma in Molecular Biology, DSMA	Teaching
		1st Year M.MedSc (Microbiology) DSMA	Teaching
2.	Dr. Myat Htut Nyunt	Training Workshop on molecular analysis of drug resistant malaria	Teaching & Demonstration
3.	Daw Ohnmar Lwin	1st year Diploma in Molecular Biology, DSMA 1st Year M.MedSc (Microbiology) DSMA	Demonstration
4.	Dr. Phyu Win Ei	1st year Diploma in Molecular Biology, DSMA 1st Year M.MedSc (Microbiology) DSMA	Teaching & Demonstration
5.	Dr. Hnin Ohnmar Soe	1st year Diploma in Molecular Biology, DSMA 1st Year M.MedSc (Microbiology) DSMA	Teaching & Demonstration
6.	Dr. Zayar Han	1st year Diploma in Molecular Biology, DSMA 1st Year M.MedSc (Microbiology) DSMA	Teaching & Demonstration
7.	Daw Mi Mi Htwe	1st year Diploma in Molecular Biology, DSMA 1st Year M.MedSc (Microbiology) DSMA	Demonstration