

BACTERIOLOGY RESEARCH DIVISION

Deputy Director & Head	...	Dr. Mya Mya Aye MBBS, MMedSc, PhD(Microbiology) (UM1)
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Research Assistant (3)	...	Daw Aye Yin Shwe BA(Geography)(DU) ... Daw Hay Mar Win BA(History)(EYU)
Laboratory Attendant	...	Daw Saw Nan Wai

The Bacteriology Research Division was engaged in the following research activities on acute respiratory, bacteriological aspects on therapeutics and environmental health. Research with direct implication for effective control of diseases was being focused. One of the main areas of research was the detection of emergence of drug resistant organisms in clinical samples of the patients.

RESEARCH PROJECTS

1. COMMUNICABLE DISEASES

1.1. CHOLERA

1.1.1. Molecular characterization of *Vibrio cholerae* in Myanmar (2015)

This study was carried out in collaboration with Osaka University, Japan to determine the genotypic characteristics of *Vibrio cholerae* strains from Myanmar and to analyse the genotypic characteristics of *V. cholerae* isolates in the recent years. *Vibrio cholerae* O1 El Tor has dominated the seventh cholera pandemic since early 1960's. For the recent two decades, variants of *V. cholerae* O1 El Tor that produce classical cholera toxin have emerged and spread globally, replacing the prototypic El Tor biotype. Pulsed-field gel electrophoresis (PFGE) revealed nine different patterns among 34 *V. cholerae* O1 isolates in Yangon, 2012, and multiple-locus variable-number tandem-repeat analysis (MLVA) yielded 13 different types of isolates. In 2013, 23 out of 24 isolates exhibited identical pulsotype Y7, and a total of eight types were identified by MLVA. Most cholera cases in children in these two years were caused by *V. cholerae* O1 belonging to identical or closely related types. In 2014, among tested 60 strains, new types of PFGE/ MLVA were recognized and previous particular types vanished. The current 2015 Yangon isolates (n=25) were found to be El Tor variant carrying the El Tor *rstR* gene and the classical cholera toxin (CT) B subunit (*ctxB*) gene. The occurrences of cholera in children in 2013 may have been related to persistent transmission of a clone from 2012. However, in 2014, an outbreak was caused by a different clone of *V. cholerae* O1. The 2015 Yangon isolates were classified into early Wave 3 type of seventh cholera pandemic, and nowadays they have replaced Wave 1 (prototypic El Tor biotype) and Wave 2 strains. Our data provide important initial insights into the genetic backgrounds of recent Yangon isolates of *V. cholerae* O1. Epidemiological surveillance linked to laboratory investigations is important to minimize the risk of *V. cholerae* infection.

1.2. Phenotypic and genotypic characterization of carbapenemase producing *Enterobacteriaceae* (Distribution of Carbapenem-Resistant *Enterobacteriaceae* in Yangon General Hospital, Myanmar)

This study was carried out in collaboration with Osaka University, Japan to determine the phenotypic and genotypic characterization of carbapenemase producing *Enterobacteriaceae*. The clinical samples including urine, pus, sputum, throat, wound and blood submitted to Microbiology Laboratory at Yangon General Hospital were subjected to determine the bacteriological identification and antimicrobial susceptibility testing; which were determined by using VITEK2 automatic system (SysmexbioMerieux, Marcy l'Etoile, France) at the Microbiology laboratory, Yangon General Hospital. *Enterobacteriaceae* isolates showing minimum inhibitory concentration (MIC) levels of 2 µg/mL or more for meropenem were transferred to Bacteriology Research Division, Department of Medical Research and Osaka University, Japan, for further phenotypic and molecular analysis. Of 29 isolates tested, 14 isolates were reconfirmed to show the higher MIC levels to both imipenem and meropenem. There were 10 isolates of *Escherichia coli*, 2 isolates of *Klebsiella pneumoniae* and 2 isolates of *Enterobacter cloacae*. Clinical specimens were mostly blood, followed by urine, sputum and peritoneal fluid. Sources of the pathogens were distributed to various clinical wards; hematology ward (8 cases), medical ward (2 cases), intensive care unit (2 cases) and surgical ward (2 cases). MIC levels of the carbapenems ranged 2 to more than 16 µg/ml. All these CRE-suspected isolates were subjected to PCR analysis to screen for major carbapenemase genes (*bla_{NDM}*, *bla_{KPC}*, *bla_{IMP}*, *bla_{OXA-48}*). The PCR products were purified and subsequently sequenced by the 3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA). The results demonstrated that all strains harbored *bla_{NDM}* genes and the nucleotide sequencing showed *bla_{NDM-5}* (5 strains), *bla_{NDM-1}* (4 strains), *bla_{NDM-4}* (3 strains) and *bla_{NDM-7}* (1 strain). Results of PCR for the other major carbapenemase genes were all negative.

1.3. ACUTE RESPIRATORY INFECTION

1.3.1. Bacterial, viral and atypical pathogens associated with acute respiratory infections and their clinical characteristics among children admitted to Yangon Children Hospital: Bacteriological findings

Acute respiratory infections (ARI) in children comprises a complex group of illnesses of different aetiologies, clinical presentations and degree of severity and is one of the leading causes of morbidity and mortality in children. This study was carried out to determine bacterial, viral and atypical pathogens associated with acute respiratory infections and their clinical characteristics among children in Myanmar. Nasopharyngeal swab samples were collected from 390 children (one month to 12 years of age) with acute respiratory infection attending Yangon Children Hospital. The demographic and clinical information were recorded in a proforma. Both molecular and conventional viral/bacterial identification methods of bacterial pathogens for ARI were carried out in Bacteriology Research Division, Department of Medical Research. Analysis of demographics, clinical characteristics and outcome of different pathogens responsible for hospitalized ARI cases were conducted at Clinical Research Division, Department of Medical Research. Of 390 samples tested by both conventional culture and multiplex polymerase chain reaction, bacterial pathogens detected were *Staphylococcus aureus* (8.7%, (34/390), *Haemophilus influenzae* (2.5%, 10/390), *Streptococcus pneumoniae* (4.4%, 17/390), coagulase negative Staphylococci (13.3%, 52/390), *Moraxella catarrhalis* (4.4%, 17/390) and *Streptococcus viridians* (4.6%, 18/390). Atypical bacterial pathogens detected were *Chlamydia pneumoniae* (2.3%, 9/390), *Legionella pneumoniae* (2.1%, 8/390) and *Mycoplasma pneumoniae* (2.8%, 11/390). The findings on wide range of etiological agents of acute respiratory infections and their clinical characteristics are useful in timely diagnosis and treatment of paediatric acute respiratory infections in Myanmar.

2. ENVIRONMENTAL HEALTH

2.1. Microbiological evaluation of indoor air quality in two major hospitals in Yangon

This study was carried out to determine the microbiological quality of indoor air of two tertiary hospitals from June 2014 to May 2015. Indoor air samples from 13 wards/unit of Yangon Children Hospital (YCH) and 10 wards/unit of Yankin Children Hospital (YKCH) were collected by using the settled plate techniques to enumerate bacterial and fungal isolates, to identify the pathogenic bacteria and to determine the antimicrobial sensitivity profile of isolated pathogenic bacteria. Thirteen bacterial and fungal species were cultured from the hospital air and their average total airborne microbial count from different wards showed wide range from 37.5– 690.75 cfu/dm²/hr and 54–304.5 cfu/dm²/hr. The counts were compared with the standard acceptable limit. The unacceptable increased aerobic colonies counts of 630 and 509 colony forming unit (cfu/dm²/hr) were observed from YCH Surgical ward 1 and 2 respectively. Gram positive bacterial load of 1555.5cfu/dm²/hr and 710.25 cfu/dm²/hr were highest in winter in YCH and YKCH. Gram negative bacterial load of 63 cfu/dm²/hr and 59.25 cfu/dm²/hr were highest in the rainy season in YCH and YKCH. Fungal load of 27.75 cfu/dm²/hr was found to be highest in winter in YKCH and highest load of 120 cfu/dm²/hr detected in the rainy season in YCH. The most commonly isolated bacteria were *Staphylococcus aureus* and *Escherichia coli*. *Staphylococcus aureus* (n=55) isolates were found to be totally susceptible to Piperacillin/tazobactam(100%), highly susceptibility to Cefoxitin(98%), Amikacin(94%), Gentamicin(83%), Cefoperazone/sulbactam(81%), moderately sensitive to Ceftriaxone(77%), Ciprofloxacin(74%), Vancomycin(73%), Amoxicillin-clavulanic acid (72%), Chloramphenicol (71%) and resistant to Erythromycin(33%), Azithromycin (32%) and Ampicillin (31%). *E.coli* (n=24) isolates were highly susceptible to Meropenem(80%), Gentamicin, Imipenem, Amikacin (70% each) and moderately susceptible to Cefoperazone sulbactam (64%), Ceftazidime, Piperacillin/tazobactam (60% each) ,Ciprofloxacin(58%), and resistant to Amoxicillin-clavulanic acid (57%), Ampicillin (45%), Ciprofloxacin, (42%). Among 25 *Staphylococcus aureus*, 17 were Coagulase negative Staphylococci, 3 were methicillin resistant *Staphylococcus aureus* (MRSA) isolates and 4 were methicillin resistant *coagulase negative Staphylococci* (MRCoNS) isolate respectively. The research findings were beneficial for the microbiological evaluation of indoor air quality in two tertiary children hospitals in Yangon and it would also be valuable in implementing strategies for improving infection control measures in hospital environment.

2.2. Bacterial analysis of drinking water in selected suburb area

This study was cross sectional laboratory based analytical study to assess the bacteriological quality of household drinking water in selected suburb area in North Okkalapa Township, Yangon, by using simple random sampling method during 2015. There were 102 households involved in this study and household water samples were collected from two sources, one from storage site and another from drinking pot (end point of consumption). From 204 water samples, 45 *Escherichia coli* (22%), 36 *Enterobacter spp.* (17.6%), 31 *Citrobacter spp.* (15.2%), 9 *Vibrio cholerae* (4.4%), 4 *Kiebsiella spp* (2%), 3 *Pseudomonas spp.* (1.5%) and 1 *Salmonella spp.* (0.49%) were isolated and identified by conventional culture method. Antibiotic susceptibility tests were done by using modified Kirby Bauer disc diffusion method and the Clinical and Laboratory Standards Institute (CLSI) zone size interpretation were also used to identify susceptible and resistant isolates. It was found that the isolated bacteria were mostly susceptible to cefoperazone sulbactam, norfloxacin, levofloxacin and co-trimoxazole. However, they were highly resistant to ampicillin followed

by gentamicin. Serotyping of *Escherichia coli* isolates were also done by using specific antisera and the result revealed that 3 *Enterotoxigenic Escherichia coli* (ETEC) (2.9%), 5 *Enteropathogenic Escherichia coli* (EPEC) (5%), 2 *Enteroinvasive Escherichia coli* (EIEC) (2%) and 3 un-typable (3%) from drinking pot. From household water samples storage site, 18 *Enterotoxigenic Escherichia coli* (ETEC) (17.6%), 6 *Enteropathogenic Escherichia coli* (EPEC) (5.9%), 1 *Enteroinvasive Escherichia coli* (EIEC) (1%) and 7 un-typable (6.9%) were also detected. The present study provided the information on microbiological quality of drinking water in the study area and may assist in developing intervention programmes for household drinking water storage and treatment strategies.

3. TRADITIONAL MEDICINE

3.1. Evaluation of anti-bacterial activities of *Mimusops elengi* Linn. and *Aloe vera* Linn. on oral pathogens

Different extracts of 2 medicinal plants were tested for *in-vitro* antibacterial activity by using agar disc diffusion technique. The minimum inhibitory concentrations (MIC) of the extracts with the most significant antibacterial activity were evaluated by both tube dilution and plate dilution method. The bacterial strains tested were *Candida albicans*, *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas spp.* Of four bacterial strains tested, the different extracts of *ခေရ (Mimusops elengi Linn.)* and *သူးစောင်းလက်ပတ် (Aloe vera Linn.)* have antibacterial activity on *Pseudomonas spp* and *Staphylococcus aureus*. The ethanol extract of *ခေရ (Mimusops elengi Linn)* showed the lowest MIC (≥ 12.25 mg/mL) on *Staphylococcus aureus* and aqueous extract of *ခေရ (Mimusops elengi Linn.)* showed the lowest MIC (≥ 12.25 mg/mL) on both *Pseudomonas spp* and *Staphylococcus aureus*. Both ethanol and aqueous extracts of *သူးစောင်းလက်ပတ် (Aloe vera Linn.)* has antibacterial activity on *Pseudomonas spp* with the lowest MIC of (≥ 12.25 mg/mL) and (≥ 50 mg/mL) respectively. The ethanol, methanol and aqueous extract of *သူးစောင်းလက်ပတ် (Aloe vera Linn.)* were found to have antibacterial activity with the lowest MIC of (≥ 12.25 mg/mL), (≥ 25 mg/mL), (≥ 25 mg/mL) respectively on *Staphylococcus aureus*.

SERVICES PROVIDED

ACADEMIC

Sr.	Name	Course	Responsibility
1.	Dr. Wah Wah Aung	PhD (Microbiology) MMedSc (Microbiology)	Thesis Supervision and Examiner Teaching, Thesis Supervision and Examiner
2.	Dr. Mya Mya Aye	MMedSc (Microbiology) MMedTech (Medical Laboratory Technology)	Thesis Supervision and Examiner Teaching
3.	Daw Thuzar Myint	MMedSc (Microbiology) MMedTech (Medical Laboratory Technology)	Teaching Teaching

LABORATORY

Sr.	Name of tests	Total Number Tested
1	Determination of TB IgG antibody by ELISA	50

BACTERIOLOGY RESEARCH DIVISION (POL)

Deputy Director & Head	...	Dr. Saw Myat Thwe MBBS, MMedSc(Pathology)(UM2)
Research Officer	...	Dr. Khine War Kyaw MBBS(UM2)
	...	Dr. Nwe Nwe Tun MBBS(UMM)
	...	Dr. Khin Moh Moh Htun MBBS(UMMG)
Laboratory Incharge	...	Daw Mie Mie Lwin BSc(Botany)(MU)
Research Assistant (2)	...	Daw Chaw Su BSc, MSc(Chemistry)(MU)
	...	Daw Tin Zar Aye BSc, MSc(Microbiology)(YU)
	...	Daw Cherry Kyaw Win BSc, MSc(Microbiology)(MU)
Research Assistant (3)	...	Daw San San Shwe BSc(Chemistry)(UDE)
	...	Daw Lai Yin Win BSc(Physics)(MU)
	...	Daw Phyo Mon Oo BSc(Zoology)(MU)
Laboratory Attendant	...	Daw Wai Wai Oo BA(Myanmar)(UDE)

Bacteriology Research Division has been actively engaged in conducting a number of research projects in areas of infectious diseases. The division provides academic services and collaborates with other universities for postgraduate students.

RESEARCH PROJECTS

1. COMMUNICABLE DISEASES

1.1 DIARRHOEA

1.1.1 Antimicrobial resistance of *Vibrio cholerae* among patients admitted for acute watery diarrhoea in hospitals of Mandalay City

Acute diarrhoea defined as an increased frequency of defecation (3 or more times per day or at least 200 g of stool per day) lasting less than 14 days, may be accompanied by nausea, vomiting and abdominal cramping. Acute diarrhoeal diseases may occur in all ages. Acute diarrhoea can be caused by a number of different agents such as viruses mostly caused by Norovirus, Rotavirus, Adenovirus, or Astrovirus, bacteria, or parasite. Rotavirus diarrhoea is an acute infection primarily of children less than 2 years of age, characterized by watery stools and vomiting. Cholera remains one of the great epidemic diseases of the tropical world. A case of cholera is confirmed when *Vibrio cholerae* O1 or O139 is isolated from any patient with diarrhoea. Cholera is an extremely virulent disease that affects both children and adults. The prominent clinical feature of cryptosporidiosis is diarrhoea, which is mild and self-limited (1-2 weeks) in normal persons but may be severe and prolonged in immunocompromised person. A total of 390 participants at (550) and (300) Bedded Children Hospitals (Mandalay), Mandalay General Hospital and (300) Bedded Teaching Hospital (Mandalay) from May 2015 to August 2015 were enrolled in this study and analyzed the data. In this study, 104 patients (26.7%) out of 390 participants revealed infected with *Vibrio cholerae* according to their stool culture result. Twenty nine out of 100 under five years age patients (25.4%) revealed Rota virus (detected by using SD bioline Rota virus test device). Fifty one cases revealed *Cryptosporidium parvum* infection all immunocompromised persons. Antibiotic sensitivity pattern showed resistant to Tetracycline, Cotrimoxazole and Erythromycin while sensitive to Doxycycline, Ciprofloxacin and Chloramphenicol.

1.2 TUBERCULOSIS

1.2.1 Comparative evaluation of Microscopic Observation Drug Susceptibility (MODS) assay and solid culture of *Mycobacterium tuberculosis* among patients with suspected pulmonary tuberculosis

(Please refer to the Annual Report of Virology Research Division)

SERVICES PROVIDED

ACADEMIC

Sr. No	Name	Course	Responsibility
1.	Dr. Saw Myat Thwe	Postgraduate Studies for the Degree of Master of Myanmar Traditional Medicine	Technical Advisor

LABORATORY

Sr. No	Laboratory Tests	Tested samples
1.	Histopathology examination for sub-acute toxicity activity of Thawy-toe-kya-say	7 samples
2.	Antibacterial activity (မုယားကြိုး)(ရှားစောင်းလက်ပတ်) <i>Boerhavia diffusa</i> , <i>Alysicarpusa monilifer</i> , <i>Carcia papaya</i>	5 samples