



## **AIMS OF THE JOURNAL**

- ❖ To serve as an important medium for the publication of original research work in the field of medical science and health research, thus filling gaps in health knowledge for effective utilization of research findings
- ❖ To disseminate recent basic, applied and social research findings among health personnel of different strata for enhancing nation-wide health development in Myanmar
- ❖ To offer current medical knowledge and updated scientific information obtained from research to health professionals for better and appropriate health care management

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## EDITORIAL

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The Myanmar Health Sciences Research Journal is always trying to share an advanced knowledge of health research information to the public with the vision of achieving a healthier nation through application of research findings. As the journal significantly contributes to the improvement of the health of the people of Myanmar, the leading article of this issue discusses about Food-borne diseases (FBD), an important public health problem especially in developing countries. The importance of different food-borne diseases varies among countries depending on foods consumed, food processing, preparation, handling, storage techniques employed, and sensitivity of the population. The increased incidence of food-borne illness occurs due to changes in eating patterns, the increasingly longer interval between processing and consumption of foods and the increasing habit of eating food prepared outside the home.

Among a variety of ready-to-eat food, sushi which is a traditional Japanese food prepared from raw and cooked fish and soured rice, has become popular in our country available at the supermarket as a frozen or cooled package. As sushi itself is perishable and it involves a significant degree of manual handling during preparation, it is normally regarded as a potentially hazardous food which are foods that are able to support the growth of pathogenic microorganisms and need to be maintained at certain temperatures to minimize this growth and prevent the formation of toxins in the food.

In this article, isolating and identifying bacteria in different types of sushi from various outlets in Yangon and detecting toxin production from isolated *Staphylococcus aureus* were studied for the consumers' safety. It was found that majority of supermarket samples were more likely to have high coliform and fecal coliform count and *Esch. coli* contamination and seafood sushi was more likely to be contaminated with *Esch. coli*. Highest *Esch. coli* count was detected in meat sushi especially processed meat such as ham. *Staphylococcus aureus* and *Bacillus cereus* were detected from fresh sushi of restaurant and sushi bar samples. The findings highlighted to provide information and education for public awareness of food safety by keeping the correct storage temperature, handling practice and environmental sanitation.

The other articles mentioned in this issue also provide invaluable health research information about wide view on hepatitis B infection, safety measures on agricultural pesticide utilization, antimicrobial resistance patterns of malaria vector, septic abortion cases, effectiveness of local anti-snake venom administration, brucellosis, knowledge on reproductive health, diabetes mellitus, etc. Therefore, the editorial committee sincerely hopes that the readers will have pleasure of reading this issue. Finally, we would like to appreciate authors for their great efforts in preparing respective research papers in this issue.

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## Aspects of Hepatitis B Virus Infection in Myanmar

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### *Introduction*

Hepatitis B virus infection is caused by hepatitis B virus (HBV), a double-stranded, enveloped DNA virus of the Hepadnaviridae family that replicates in the liver and causes hepatic dysfunction. Worldwide, there are more than two billion people infected with HBV out of which 350 million individuals are chronically infected with hepatitis B virus.<sup>1</sup>

Hepatitis B virus (HBV) infection is one of the major global public health problems and the 10<sup>th</sup> leading cause of death. HBV related hepatocellular carcinoma (HCC) is the 5<sup>th</sup> most frequent cancer worldwide. HBV is distributed worldwide, but its prevalence varies significantly between different populations of the world. Based on the prevalence of HBV surface antigen (HBsAg) carrier rate in the general population, sub-Saharan African, East Asian and Alaskan populations are classified as having high HBV endemicity (HBsAg carriage >8%), while the populations of southern parts of Eastern and Central Europe, the Amazon basin, the Middle East, and the Indian subcontinent are classified as intermediate HBV endemicity (HBsAg carriage 2-7%), and the populations in western and northern Europe, North America, and Australia are classified as low HBV endemic (HBsAg carriage <2%) regions.<sup>2</sup>

HBV infection in the Asia-Pacific region is among the highest in the world, and chronic HBV infection in most of the countries of the Asia-Pacific region is high (>10% prevalence).<sup>3</sup> Approximately 100 million hepatitis B virus carriers, more than 5.6% of the total population, live in

countries of the South-East Asia Region. More than 300,000 people are estimated to die each year due to the chronic consequences of hepatitis B, particularly cirrhosis and liver cancer.<sup>4</sup> The Republic of the Union of Myanmar has been listed among the top ten countries with the highest incidence of hepatocellular carcinoma due to hepatitis B and C. Numerous studies have been carried out on the different aspects of hepatitis B infection in Myanmar. This article is a review of some of the research projects conducted by the scientists of Myanmar over a period of six decades.

### *HBV infection in Myanmar*

The earliest data on viral hepatitis were based on the field experiences of Min Sein who was stationed at a Field Hospital in the Indo-Burma Border in the 1940s. The principal investigator had observed that infective and post-arsenical hepatitis was the second largest category of disease after malaria.<sup>5</sup> This was followed by a study on 63 viral hepatitis patients admitted to Ward 1 of Rangoon General Hospital in 1957, in which the author mentioned that two immunologically distinct forms of hepatitis, namely the infectious and serum hepatitis were detected among the patients.<sup>6</sup> The magnitude of the problem of hepatitis B infection in Myanmar was reported in a field study carried out on 1896 subjects residing in 522 households in lower (Bago Township) and upper Myanmar (Sagaing Township) which elicited that the overall prevalence of HBsAg was not significantly different between urban (9.5%) and rural populations (10.39%).<sup>7</sup>

The development of serological assays to detect hepatitis B surface antigen (HBsAg)

has played a major role in the diagnosis of hepatitis B virus (HBV) infection. Serological investigation of 162 patients with viral hepatitis admitted to the Infectious Diseases Hospital, Yangon, during 1981-82 revealed that 39.5% were hepatitis B, 3.1% were hepatitis A and 57.4% were due to non A, non B hepatitis.

In a similar study also conducted in 1982, from a total of 519 patients admitted to the Infectious Diseases Hospital, 16.2% and 6.9% had serological evidence of hepatitis B and hepatitis A, while 76.9% were due to non A, non B hepatitis.<sup>8</sup> Subsequent studies carried out among different population groups revealed HBsAg carrier rate of 10-12%<sup>9</sup> which was regarded as the national figure for HBV prevalence in the country.

Community-based studies had revealed that certain areas in Myanmar had a relatively higher HBsAg positivity rate while some areas had lower prevalence than the national figure of 10-12%. The HBsAg prevalence rate was 20.5% among 420 residents of 12-65 years from Namtee, a town in the Kachin State which is situated in the northern part of the country,<sup>10</sup> but in contrast, the HBsAg seropositivity was 4.9% in Tamu Township which is situated in the north-west border region of the country.<sup>11</sup>

#### *Studies on transmission of HBV*

In countries where HBV is endemic, most infections result from vertical transmission from mother to child in the peripartum period or from infection in early childhood. Hepatitis B is transmitted through blood and bodily fluids, and an infected woman can transmit the infection to her baby during the delivery process.<sup>12</sup>

Different research studies had shown that HBV infection is highly prevalent in Myanmar, with 10-12% HBsAg carrier rate, mainly due to vertical transmission from infected mothers to the babies. After HBV infection 70-80% of babies and 5-10% of adults become chronically infected which can lead to cirrhosis or malignancy.<sup>8</sup> Findings from a study on perinatal trans-

mission of HBV from HBV carrier mothers to their infants showed that HBV was transmitted in 24% of babies born to HBsAg-positive mothers and 61.6% of HBsAg and HBeAg-positive mothers.<sup>13</sup>

A similar study was carried out on 20 babies delivered from 16 HBsAg-seropositive mothers and 4 HBsAg and HBeAg-positive mothers at Thingyangyun Sanpya Hospital and South Dagon Hospital. These babies were followed up at 5 to 10 months. Fifty percent of babies delivered from HBsAg and HBeAg-positive mothers were found to be positive for both HBsAg and HBeAg at follow-up. Similarly, 15% of babies born to HBsAg-positive mothers were seropositive for HBsAg. At 5-10 months of age, 85% of babies born to HBsAg-positive mothers and 50% of the babies from HBsAg and HBeAg-positive mothers were found to be still seronegative for HBsAg.<sup>14</sup> HBV is transmitted by percutaneous or mucosal exposure to infectious blood or body fluids. Hepatitis B surface antigen (HBsAg) has been identified in almost all body fluids from infected persons including saliva, urine and breast milk. Although HBsAg has been detected in multiple body fluids, only serum, semen, and saliva have been demonstrated to be infectious. HBV is concentrated most highly in serum, with lower concentrations in semen and saliva.<sup>15</sup>

Blood and saliva samples were collected from 29 known healthy carriers of hepatitis B surface antigen (HBsAg) and 3 apparently normal persons who were negative for HBsAg. Findings showed that 53.57% of the saliva samples from the carriers were positive for HBsAg. HBsAg was detected in the saliva of 85.7% of persons with high titer HBsAg (1:32) determined by Counter-Immuno-Electrophoresis (CIEP) method compared to 20% in persons with a titer of 1:2.<sup>16</sup> The presence of HBsAg in oral samples is significant both as a diagnostic potential and as a possible route of transmission.

Partners or individuals living in close contact with an HBV infected person are

listed among the high-risk individuals. Horizontal transmission of HBV can occur among infants, children and elementary school students who live with infected household members.<sup>12</sup> The risk of HBV infection in family contacts of HBsAg carriers was highlighted in a study in which 50 HBsAg carriers (25 attendees of Hepatitis Carrier Clinic at the Department of Medical Research, Lower Myanmar, and 25 HBsAg-positive cases from the Liver Unit and Out-patients Department (OPD) of Yangon General Hospital were chosen as index cases and 50 HBsAg-negative persons were recruited as controls. Both the subjects and controls were tested for hepatitis B markers (HBsAg and HBeAg) and the respective family members were tested for HBsAg. HBsAg prevalence was 30% among the offspring of HBsAg-positive mothers, 21.4% among siblings of carriers, 45.5% in children born from HBsAg and HBeAg-positive mothers, 21.1% in HBsAg-positive but HBeAg-negative mothers. The study highlighted that both vertical and horizontal transmissions were significant in intra-familial spread.<sup>17</sup>

#### *Molecular studies of HBV*

Although the serological assays to detect hepatitis B surface antigen (HBsAg) have played a major role in the diagnosis of hepatitis B virus (HBV) infection, serological profiles can at times be atypical or ambiguous. Many studies had demonstrated that HBV infection may persist in the absence of circulating HBsAg.<sup>18, 19</sup> Some of the difficulties may be overcome by HBV-DNA testing, which may elucidate an individual's true hepatitis B status.<sup>20</sup> The occult or silent infection is mostly seen in individuals positive for antibodies to HBV antigens such as anti-HBc with or without anti-HBs, but also occurs in considerable number of patients negative for all serum markers.<sup>21</sup>

Despite a number of studies documenting hepatitis B virus (HBV) infection in the absence of hepatitis B surface antigen

(HBsAg), a causal relationship between silent HBV infection and liver disease remains difficult to establish. Data documenting HBV infectivity or re-infection in the absence of detectable HBsAg support the hypothesis that in some of these cases, HBV is undergoing low-level replication in the liver, in several situations including: (1) chronic liver disease, alcoholic liver disease, hepatocellular carcinoma; (2) viral reactivation following cancer chemotherapy or immunosuppression and (3) transmission via transfusion or from human serum to chimpanzees.<sup>22</sup> In one study, Nested Polymerase Chain Reaction method was used and low levels of HBV genome were detected in 23 vaccinees who failed to respond to a booster dose of plasma-derived hepatitis B vaccine locally produced at the Department of Medical Research (Lower Myanmar). Silent or occult infection was detected in 8.7% of 23 non-responders.<sup>23</sup>

A molecular-based epidemiological survey of various hepatitis viruses (HBV, HCV, HEV, and GBV-C/HGV) was carried out on 403 subjects (213 healthy persons and 190 patients with liver diseases). Although the hepatitis B infection was significantly higher among the subjects with various liver diseases compared to healthy controls (30% versus 8%), the infection rates of HCV (27% in both groups) and GBV-C/HGV (8% versus 11%) were comparable in both groups. The most common hepatitis B genotype was C (77%), genotype 3b of HCV (67%), and genotype 2 of GBV-C/HGV (67%). Anti-HEV immunoglobulin G (IgG) was detected in 32% of 371 subjects from Yangon.<sup>24</sup>

In another study, Nested Polymerase Chain Reaction (Nested PCR) method was used for detection of HBV-DNA in 29 sera samples of known hepatitis B carriers with high titres of HBsAg (RPHA titer 512 and above) determined by In-house Reverse Passive Haemagglutination test kit. Two-stage PCR was based on the X gene of the hepatitis B virus. Detection of a 118 base pair DNA band indicated that the serum



samples were positive for HBV-DNA and still undergoing active viral replication. In this study, 100% (29 out of 29) of hepatitis B carriers with high HBsAg titer had concurrent HBV-DNA in the sera.<sup>25</sup> In another study, HBV-DNA was detected in 33.33% of 39 paraffin embedded liver tissue samples of primary hepatocellular carcinoma (HCC) cases admitted to the Surgical Units of the Yangon General Hospital.<sup>26</sup>

#### *Studies on HBV genotypes*

Hepatitis B virus has been classified into eight genotypes (A-H) based on genome sequence divergence of >8% in the entire HBV genome which consists of approximately 3200 base pairs.<sup>27, 28</sup>

Distribution of HBV subtypes and genotypes varies with different geographical areas and in different ethnic groups. Genotype A is common in Northwest Europe, sub-Saharan Africa, India and United States, B and C are frequent in Southeast Asia, Japan and Oceania, and D is common in the Mediterranean countries. Genotype E is restricted to Africa, and F is found mainly in Central and South America. Genotype H is detected in patients from Nicaragua, Mexico and California.<sup>29</sup> Throughout the years, studies on correlation of hepatitis B genotypes with HBeAg clearance, liver disease and response to interferon therapy have been done by different investigators. Studies had shown that HBV genotype B is associated with better response to interferon therapy than genotype C.<sup>30, 31</sup> Studies conducted on Chinese patients with chronic hepatitis B found that genotype C was more prevalent in patients with cirrhosis.<sup>32, 33</sup>

In spite of the high percentage of HBsAg-positive individuals in Myanmar, few data exist on the hepatitis B genotypes and subtypes prevalent in the country. Among the few was a laboratory-based study on the genotyping of 100 randomly selected HBsAg carriers attending the Hepatitis Carrier Clinic, Department of Medical

Research (Lower Myanmar). Genotyping was carried out by Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (RFLP) analysis of the PCR product. HBV-DNA was detected in 62.4% of 93 HBsAg carriers. Genotype C was the most dominant (50%) among the HBsAg carriers in Yangon, and the remaining types included genotypes A, B and D.<sup>34</sup> Another laboratory-based study was carried out on 46 HBsAg-positive subjects from Muse, a border town in the north east of Myanmar near China. Genotyping was done by Polymerase Chain Reaction and Restriction Fragment Length Polymorphism (RFLP) analysis. The most common genotype was C (82.6%), followed by genotype B (13.1%) and D (4.3%).<sup>35</sup> Findings from the two studies revealed that the most prevalent genotype in the country is genotype C, followed by genotypes B and D.

#### *Studies on HBsAg subtypes*

HBsAg contains a group-reactive *a* determinant shared by all strains of HBV and two pairs of mutually exclusive, subtype-specific determinants, *d* or *y* and *w* or *r* which are used for the identification of subtypes. Thus, there are at least four major groups into which HBsAg can be classified: *adw*, *adr*, *ayw* and *ayr*.<sup>36, 37</sup>

However, additional determinants designated the sub-determinants of *w* (*w1* to *w4*) have allowed four serotypes of *ayw* (*ayw1*, *ayw2*, *ayw3*, *ayw4*) and two serotypes of *adw* (*adw2*, *adw4*). The *q* determinant was initially described as being present on all subtypes apart from *adw4*. Later, absence of *q* was demonstrated in some *adr* subtype containing sera. Thus, *adr* strains are described as *adrq+* or *adrq-*.<sup>38</sup> The classification of HBsAg now includes nine main subtypes, namely, *ayw1*, *ayw2*, *ayw3*, *ayw4*, *ayr*, *adw2*, *adw4*, *adrq+* and *adrq-*.<sup>39</sup>

To find out the distribution of subtypes of HBsAg-positive persons in Yangon, Myanmar, subtyping of HBsAg was carried out on 103 HBsAg high-titer sera samples selected from 362 HBsAg carriers attending

the Liver Unit, Yangon General Hospital and Hepatitis Carrier Clinic, Department of Medical Research (Lower Myanmar), Yangon during the period of 2000 to 2002. HBsAg positivity was confirmed by in-house ELISA test kit and HBsAg titer was determined by Counter immunoelectrophoresis (CIEP) method. Subtyping was done by ELISA method using monoclonal antibodies for immunodeterminants, *d*, *y* and *r*. The most prevalent subtype was *adr* (93.2%) followed by *adw* (4.85%) and *ayw* (1.94%).<sup>40</sup> Although the most prominent HBsAg subtype in Myanmar is *adr* and similar to the most common subtype in Thailand<sup>41</sup> and among the Malay population in Malaysia,<sup>42</sup> it is different from the HBsAg subtypes prevalent in Singapore (*adw*)<sup>43</sup> and Indonesia (*adw*).<sup>44</sup>

Many studies have been carried out by various researchers on different aspects of hepatitis B infection as it is a major health problem in Myanmar and regarded as a priority disease in the National Health Plan of the country. This article is a compilation of some of the research findings carried out in the country, with the objective of providing information to the scientists, health care personnel, administrators and decision makers.

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## Bacteriological Contamination of Sushi from Various Outlets in Yangon

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Ninety-two samples of meat, seafood, vegetable, tofu and egg sushi from different outlets such as restaurants, sushi bars and supermarkets in Yangon were examined for bacteriological contamination. Aerobic Plate Counts were not so different with all counts falling into satisfactory grade for consumption. Sushi was found to be unsatisfactory for total coliform group comprising of 44% of restaurant samples, 55% of sushi bar samples and 91.7% of supermarket samples. Sushi was also unsatisfactory for fecal coliforms comprising of 33% of restaurant samples, 25% of sushi bar samples and 92% of supermarket samples. It was found that 51.1% of sushi samples were contaminated with *Escherichia coli* and among them 68% had high count and unsatisfactory grade. Different virulence groups included EAEC (41.6%), EIEC (27%), EPEC (25%), EHEC (5%) and ETEC (1.6%). *Staphylococcus aureus* was found in 7.6% of total sushi samples and isolates were tested for staphylococcal enterotoxin by reverse passive latex agglutination test. However, staphylococcal enterotoxin was not detected. Some sushi samples (4.3%) contained *Bacillus cereus*. The pH of the sushi rice appears to be variable and does not seem to affect pH of ingredients to sustain bacterial growth. In conclusion, majority of supermarket samples were more likely to have high coliform and fecal coliform counts and *Esch. coli* contamination and seafood sushi was more likely to be contaminated with *Esch. coli*. Highest *Esch. coli* count was detected in meat sushi especially processed meat such as ham. *Staphylococcus aureus* and *Bacillus cereus* were detected from fresh sushi of restaurant and sushi bar samples.

## INTRODUCTION

Food-borne diseases (FBD) of microbiological origin are becoming an important public health problem especially in developing countries. The importance of different food-borne diseases varies among countries depending on foods consumed, food processing, preparation, handling, storage techniques employed, and sensitivity of the population.<sup>1</sup>

People demand a wider variety of foods than in the past. Changes in eating patterns, the increasingly longer interval between

processing and consumption of foods and the increasing prevalence of eating food prepared outside the home all contribute to the increased incidence of food-borne illness. Popularity of culturally diverse ready-to-eat foods and insufficient access to safe water and facilities for safe food preparation are creating an environment for both known and emerging new food-borne diseases. In this study, sushi was chosen as it represents the challenge associated with culturally diverse foods. Sushi, a Japanese traditional food, has become popular in Myanmar.

Sushi is a convenient ready-to-eat food and can be purchased from the supermarkets as a frozen or cooled package, from sushi bars as a display product inside an enclosed cabinet and from restaurants. Sushi is prepared with raw fish or other seafood, meat or egg in combination with rice prepared with vinegar and spices. Meat and fish for sushi preparation should meet the highest quality requirement so appropriate preparation, compliance with cold chain during production, transportation and storage are of utmost importance.

As sushi itself is perishable and it involves a significant degree of manual handling during preparation, it is normally regarded as a potentially hazardous food that are able to support the growth of pathogenic microorganisms at certain temperatures and need to be maintained at temperatures either below 5°C or above 60°C to minimize this growth and prevent the formation of toxins in the food.<sup>2</sup> Sushi (nigiri and nori rolls) can be stored for up to 12 hours at temperatures between 5°C and 15°C provided that the pH of the rice is <4.8.<sup>3</sup> According to the guidelines for the micro-biological examination of ready-to-eat-foods from Food Standards Australia New Zealand (2001), four categories such as satisfactory, marginal, unsatisfactory and potentially hazardous have been assigned to assess microbial contamination of ready-to-eat-foods based on standard plate counts, levels of indicator organisms and the number or presence of pathogens.<sup>4</sup>

Japanese sushi preparation has a long tradition involving specially trained cooks and standardized processing method. This situation is different in other countries, where it may or may not be practiced. In this study, the safety of sushi with regard to exposure of bacterial agents is studied in actual product available to the consumer.

#### *General objective*

- To study the bacteriological contamination of sushi from various outlets in Yangon

#### *Specific objectives*

- To isolate and identify bacteria in different types of sushi from various shops
- To detect toxin production from isolated *Staphylococcus aureus*

## **MATERIALS & METHODS**

A cross-sectional, laboratory-based descriptive study was carried out from January to October 2011 at Biological Toxicology Research Division and Bacteriology Research Division, Department of Medical Research (Lower Myanmar). Samples were collected from restaurants, sushi bars (or) retail outlets and supermarkets in Yangon.

The ingredients of samples were categorized as meat sushi, seafood sushi and vegetarian sushi. Sushi can be classified as category II food and the number of samples needed for meaningful analysis is 30.<sup>5</sup> However, there are some variables to be considered and 27 samples from restaurants, 41 samples from sushi bars and 24 samples from supermarkets were collected with particular focus on seafood sushi. Therefore, a total of 92 samples were taken. Food samples were transported to the laboratory in the original unopened containers within 30 minutes with the original storage conditions and processed immediately on arrival.

From each sample, 25g were weighed and transferred to sterile plastic bags containing 225 ml of Butterfield's phosphate solution and homogenized in the stomacher for 1 minute. These results in a dilution of 10<sup>-1</sup> food homogenate and serial dilutions up to 10<sup>-3</sup> of homogenate were done.

#### *Aerobic Plate Count (APC)*

From each dilution 20µl were taken each time and dropped onto plate count agar which was sectored into 3 parts and done in triplicate. Same procedure was done for other dilutions. After incubation at 37°C for 24 hours, colonies were counted and APC per milliliter was calculated.

*Enumeration, isolation and identification of coliforms, fecal coliforms and Esch. coli*

Most Probable Number (MPN) method utilizing multiple dilution to extinction in Lauryl tryptose (LT) broth and brilliant green bile broth (BGB) was used as a presumptive and confirmation test for total coliforms and EC broth was used as confirmed test for fecal coliforms and *Escherichia coli*. For *Escherichia coli* confirmation, a loopful of suspension from each gassing EC medium was taken and streaked on MacConkey agar and incubated at 35°C for 18-24 hours. Suspected typical lactose fermenting colonies showing gram-negative rods or cocci on microscopy were inoculated onto the test tube containing slanted TSI, LIA, urea agar slope, stabbed into SIM medium, MR-VP medium and Simmon's citrate medium for identification of *Escherichia coli*.

Serotyping was carried out by slide agglutination test using four polyvalent antisera; A for EPEC, B for EPEC, C for EIEC and D for ETEC, followed by 32 relevant monovalent O and O, K antisera of *Escherichia coli*.

*Isolation and identification of Staph. aureus, Bacillus cereus and Listeria*

One milliliter of 10% suspension of food homogenate was transferred to 9 ml of trypticase soy broth for enrichment and incubated at 37°C for 24 hours and sub-cultured onto blood agar and mannitol salt egg yolk agar and incubated at 37°C for 24 hours. Suspected colonies showing gram-positive cocci arranged singly, in pairs or in clusters were presumed to be *Staphylococcus* spp. and *Staphylococcus aureus* was identified by typical colonies on MSEY agar with positive catalase test, slide and tube coagulase test.

Large, rough colonies with crenated edges and  $\beta$  haemolysis on blood agar with gram-positive bacilli in short-to-long chains with spores were assumed as *Bacillus* spp. and subcultured onto nutrient agar and

biochemical tests such as SIM, Voges-Proskauer test, nitrate reduction test, gelatin hydrolysis and carbohydrate fermentation tests were done.

For listeria spp., large, rough colonies with  $\beta$  haemolysis on blood agar with short gram-positive bacilli were picked up and inoculated into TSI, SIM, MR-VP media and urea media. Catalase test was done from colonies which were subcultured onto nutrient agar.

*Toxin detection from Staphylococcus aureus*

Toxin produced by *Staph. aureus* was tested by Staphylococcal Enterotoxin Reverse Passive Latex Agglutination (SET-PRLA) for staphylococcal enterotoxins A, B, C & D.

*Isolation and identification of Salmonella and Shigella*

One milliliter of 10% suspension of food homogenate was transferred to selenite F broth for enrichment and incubated at 37°C and 43°C for 24 hours and both broths were subcultured onto Salmonella-Shigella agar and suspected colonies were identified by biochemical tests including triple sugar iron agar (TSI), lysine iron agar (LIA), sulphide indole motility (SIM), urea test, citrate test and MR-VP test.

*Isolation and identification of Vibrio parahaemolyticus*

Twenty-five grams of sample were weighed and 100 ml of 3% NaCl solution were added and homogenized in the stomacher and incubated at 37°C for 24 hours. A loopful suspension from food homogenate was cultured on thiosulfate citrate bile salts sucrose agar at 37°C for 24 hours. Round green colonies suspected of *Vibrio parahaemolyticus* were picked up and sub-cultured onto 2% alkaline nutrient agar and incubated and identified by biochemical tests including TSI, LIA, SIM, urea test, oxidase test, salt tolerance test and mannitol sugar fermentation.

## RESULTS

Aerobic plate counts of all sushi samples were positive ranging from minimum  $1.1 \times 10^4$  cfu/g and maximum  $3.3 \times 10^5$  cfu/g with a mean of  $1.8 \times 10^5$  cfu/g. According to Health Protection Agency (2009) Guidelines,<sup>6</sup> APC for satisfactory category is  $<10^6$  and in this study maximum APC was  $3.3 \times 10^5$  cfu/g so all results indicated good microbiological quality.

Sushi in this study were categorised according to the total coliform count, fecal coliform count and *Esch. coli* count and recorded as satisfactory, marginal and unsatisfactory level for consumption and these are shown in Fig. 1.

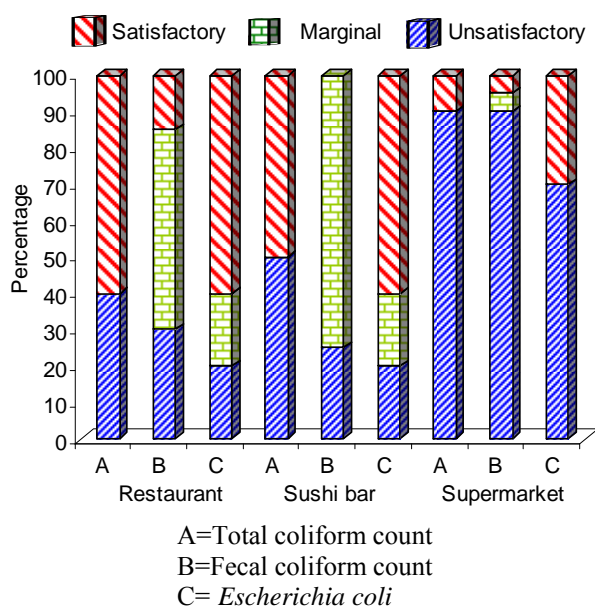


Fig. 1. Comparison of different grades of microbial contamination of sushi from different outlets

Total coliform counts of  $>1000$  MPN/g is categorized as unsatisfactory and comprised of 44% of restaurant samples, 55% of sushi bar samples and 91.7% of supermarket samples. When categorized according to the ingredients of sushi, 80% of meat samples, 54% of seafood samples and 50% of vegetable samples were unsatisfactory.

Fecal coliform counts in sushi from restaurants, sushi bars and supermarkets ranged from 3 to  $>1100$  MPN/g and detec-

tion rates were 85%, 100% and 96%, respectively. Sushi which fall into unsatisfactory category for fecal coliform count of more than 100 MPN/g comprised of 33% of restaurant samples, 25% of sushi bar samples and 92% of supermarket samples and 73% of meat samples, 43.4% of seafood samples and 55% of vegetable samples.

*Esch. coli* was isolated from 51.1% of total sushi samples. Among *Esch. coli* isolated samples, 68% was unsatisfactory as count exceeded 100 MPN/g and comprising of 22% of restaurant samples, 22% of sushi bar samples and 71% of supermarket samples.

Fourteen serogroups were detected from 60 isolated *Esch. coli*. Among them, 25 isolates were EAEC (41.6%), 16 isolates were EIEC (27%), 15 isolates were EPEC (25%), 3 isolates were EHEC (5%) and one isolate was ETEC (1.6%).

Among 92 sushi samples, 17 samples (18.5%) contained staphylococci and *Staph. aureus* was isolated from 7 samples (7.6%). *Staph. aureus* contaminated sushi comprised 6 from restaurant and 1 from supermarket.

*Bacillus* species was detected in 24 sushi (26%). *Bacillus cereus* was isolated from 4 samples (4.3%) and among them, 2 were from restaurants and 2 were from sushi bars.

*Vibrio* species were detected in 7 sushi (7.6%), however, *Vibrio parahaemolyticus* was not isolated. In this study, *Salmonella*, *Shigella* and *Listeria* species were not detected.

## DISCUSSION

In this study, indicator tests used were Aerobic Plate Count (APC), total coliform count, fecal coliform count including *Esch. coli* detection. Results showing a high APC, total coliform, fecal coliform and *Esch. coli* counts suggest contamination resulting from inappropriate processing, incomplete heating, or secondary contamination via contact with contaminated equipment such as chopping boards, knives, and serving wares.



It is recommended that the generally acceptable microbial guideline value for APC of ready-to-eat foods set at  $<10^5$  cfu/g be adapted locally until more precise microbial criteria for this food type can be developed through an appropriate scientific process.<sup>7</sup> APC can provide useful information about the general quality and remaining shelf-life of the food especially in supermarket sushi and thus highlight potential problems of storage and handling since production.

In this study, results revealed that the APCs were evenly spread among sushi samples and fall into satisfactory group. These findings were not different from other sushi survey reports in other countries. A sushi survey by New South Wales food authority (2008) also found that about 98% of samples had satisfactory APCs.<sup>2</sup>

In a sushi survey conducted in Australian Capital Territory (ACT), about 1.8% of sushi samples were found to be potentially hazardous and these were due to  $\geq 10^4$  cfu/g of *Bacillus cereus* contamination. Nearly 8% of samples were unsatisfactory as they contained 100 cfu/g *Esch. coli*, 5.5% were unsatisfactory for coagulase-positive *Staphylococci* and 3.6% were unsatisfactory for *Bacillus cereus*. *Listeria monocytogenes* was detected in 12.7% of sushi samples categorising them as marginal.<sup>8</sup>

In a German survey, microbial quality of sushi was analyzed and comparison was made between fresh and frozen sushi. APC of processed sushi was higher than that of freshly prepared sushi. The prevalence of *Esch. coli* and *Staphylococcus* was higher in fresh samples. *Listeria monocytogenes* was detected in 1.2% of samples and *Salmonella* was also detected in 1.6% of sushi samples. The study showed that sushi with fish toppings was more likely to have higher APC. The study result indicated that industrially processed sushi such as frozen sushi had higher quality than freshly prepared sushi. The quality of freshly prepared sushi strongly depends on the skills and habits of the preparation cooks.<sup>9</sup>

In this study, majority of sushi had high total and fecal coliform counts. Mean coliform counts were significantly different between restaurant (571.9 MPN/g), sushi bar (689.1 MPN/g) and supermarket samples (1036.8 MPN/g) and the difference was significant (ANOVA test,  $p=0.001$ ). Supermarket samples were more likely to have higher coliform count. Sushi which falls into unsatisfactory for total coliform group composed of 44% of restaurant samples, 55% of sushi bar samples and 91.7% of supermarket samples. This difference is significant at  $p$  value of 0.001. Sushi falling into unsatisfactory for fecal coliform group composed of 33% of restaurant samples, 25% of sushi bar samples and 92% of supermarket samples. This difference is also significant at  $p$  value of 0.002.

Regarding *Esch. coli*, 22.2% of restaurant samples, 21.9% of sushi bar samples and 70.8% of supermarket sushi were found to be unsatisfactory. These findings were different from a study which reported that *Esch. coli* detection was high in fresh sushi.<sup>9</sup>

Supermarket sushi samples indicated 3 to 4 days duration from the time of manufacture as fit-to-eat period. In this study, supermarket samples were examined one day prior to expiry date. Supermarket samples may need further inspection to determine whether food production practice or temperature of storage is a problem. In developed countries, frozen supermarket sushi were processed by companies working under a hazard analysis critical control point program which prepared food under standardized hygienic condition with a functioning cooling chain.

However, in developing countries, processing procedures were different with more manual handling. Therefore, in this study, majority of supermarket samples were more likely to have high coliform count, high fecal coliform count and high *Esch. coli* detection rate (71%) with count of unsatisfactory grade and seafood sushi was more likely to be contaminated with *Esch. coli*.

The presence of *Escherichia coli* in ready-to-eat (RTE) food products indicates the possibility of secondary contamination. On the other hand, existence of *Staph. aureus* suggests poor hygiene practices of the food handlers, cross-contamination during preparation, or improper storage.<sup>10</sup>

In this study, about 50% of gassing EC tube especially with high MPN index subcultured on to MacConkey agar had *Escherichia coli* growth and this finding was similar to Hall's study.<sup>11</sup> Half of the positive EC tubes failed to isolate *Escherichia coli* and this indicated that coliform organisms other than *Escherichia coli* gave positive EC tube.

Some restaurant meat sushi (43%), all meat sushi from sushi bars and supermarkets had unsatisfactory *Escherichia coli* count and also had highest count. These meat were ham and processed turkey. This finding was in agreement with a study from Taiwan on microbiological quality of five types of 18°C ready-to-eat food products. It was reported that food made of ham had highest incidence of coliforms (88%) and fecal coliform (80%) and the highest incidence of *Escherichia coli* (16%) was also detected in ham.<sup>12</sup>

The most important *Escherichia coli* from a food safety perspective is the verocytotoxin-producing *Escherichia coli* (VTEC). In this study, *Escherichia coli* O111 and O26 serogroups were detected. These serogroups were also detected in a study in Yangon which also reported the occurrence of serogroup O157K<sup>+</sup> in one roasted pork sample.<sup>13</sup>

In this study, 7.6% of sushi samples were contaminated with *Staph. aureus* and 4.3% of sushi samples contained *Bacillus cereus*. Microbial counts for these samples were not done in this study. Detection with quantifying is needed as consumption of levels of more than or equal to 10<sup>4</sup> cfu/g is potentially hazardous resulting in food-borne illness. Detection of *Bacillus cereus* in cooked food is due to inadequate temperature control and human contact during processing.

Overall, the study results show higher unsatisfactory rate of microbial contamination of sushi compared to other studies. However, potentially more dangerous microorganisms like *Salmonella*, *Shigella*, *Listeria*, *Vibrio cholerae* and *Vibrio parahaemolyticus* were not detected in this study.

The study found that freshly prepared sushi is relatively safe with low bacterial counts, although there is still a need for proper hygiene conditions and supporting of basic sanitary facilities. Sushi preparation needs a high hygiene standard. The quality of raw material must meet the standard. Culturally diverse food, containing raw or undercooked foods, need special care in food storage. Poor hygiene in food handlers and the lack of training in food safety are probably the most common causes of food-borne illness especially for food that are not traditional. Many people are unaware of the risk and disease contracted from eating raw or undercooked foods especially when these are not prepared properly. Guidelines should be set for preparation of foods which need special skill in preparation and which are not customarily consumed in a community; especially in developing countries where environmental sanitation is poor.

The results of this study point out that the culturally diverse ready-to-eat food may cause food safety hazard. The correct storage temperature, handling practice and environmental sanitation should be considered. Food contamination at any stage of food production, processing and delivery need to be assessed, monitored and improved. This study is intended to provide information and education for public awareness of food safety.

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## Outlooks Toward Their Assigned Jobs of Station Medical Officers

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A cross-sectional descriptive study was conducted to determine factors influencing performance of station medical officers (SMOs) from February to July 2009 in twenty-six townships of three regions of Upper Myanmar. Out of 32 SMOs, 21 (65.63%) male and 11 (34.37%) female were interviewed with structured questionnaires. Mean years of age, mean years of service, mean distance from township hospital were  $36\pm 2$  years,  $7.06\pm 1.83$  years and  $12.66\pm 6$  miles, respectively. Mean number of villages and mean number of population served by one SMO was  $34.38\pm 12$  and  $27763\pm 12049$ , respectively. Regarding the job-related factors, positive response rate of perception, satisfaction, attitude and motivation were observed at 18 (56.25%), 22 (68.75%), 18 (56.25%) and 25 (78.25%), respectively. Performance of male SMOs were 5 times better than that of female SMOs (Adjusted OR=5.5638,  $p<0.05$ ). Performance was assessed by hospital performance indicator, cooperation of health activities with local authorities and other members of health team, development of new building or operation room during years of posting in assigned hospital. Reconsiderations should be made on development of resource to upgrade infrastructure in health system especially in rural area to attain effective performance of assigned SMOs. Moreover, support and coordination of community and local authority in some station hospitals improve performance of SMOs during study period.

## INTRODUCTION

To uplift of health, fitness and education standard is the social objective of National Policy. In order to fulfill these social objectives, national health policies are currently implemented through the National Health Plan (NHP 2006-2011), in which 12 broad programme areas and 65 projects are identified under the guidance of the National Health Committee (NHC).<sup>1</sup>

These programmes and projects are implemented at the village level by Basic Health Staffs (BHS) of Rural Health Centre. Station Medical Officer (SMO) is the key person in rural hospital. At the state/regional level, public health and curative functions are exercised by Senior Medical Officer. At township level, a township medical officer is responsible for public

health curative activities. At the station level, a station medical officer is responsible for public health and curative activities. Each township has a hospital with a strength varying from 25 to 50 beds depending upon the population, one or two station hospitals and four to seven rural health centers (RHCs). Station hospitals are organized with 16-bed station hospitals and a rural health centre. A medical doctor called Station Medical Officer (SMO) is in-charge of this unit. Its main functions are more than that of a RHC. It is not just only public health function and OPD clinic but also medical care with in-patient treatment from nearby areas. SMO, the in-charge of the unit, is responsible for not only the hospital care but also rural health function like RHC.<sup>2</sup>

Population of the country is estimated at 56.5 million and population growth rate is

2.02 percent. Nearly 70% of people are living in rural areas where they can seek health care from RHCs or station hospitals run by health assistants (HA) and SMOs, respectively.<sup>1</sup>

It is believed that motivation of station medical officers can increase their performances which uplift the quality of health care especially in remote areas. This study searched for factors that motivate the station medical officers and improve their performance. It is useful information for Township Medical Officers and state and regional health officers how to improve performance of station medical officer in township, state and regional level.

*General objective*

- To find out factors influencing the performance of station medical officers, which can be applicable in health management system to produce sustainable improvement

*Specific objective*

- To assess the performance of station medical officers in different station hospitals of different areas
- To determine the factors that influence the performance of station medical officers on specific assigned jobs, public health, disease control and curative services

**MATERIALS AND METHODS**

Cross-sectional descriptive study design was used. The study was conducted during February and July 2009 in 26 townships of Mandalay, Sagaing, and Magway regions. The study involved 32 station medical officers (SMOs) from three regions, namely Wetwan, Kyaukme, Sinkaing, Kyaukse, Myittha, Myingyan, Taungtha, Windwin, Mahlaing, and Thazi from Mandalay Region; Sagaing, Chaungoo, Ahyadaw, Salingyi, Monywa, Shwebo, Yayoo, Wetlet, Kantblu,

Tazel and Butalin from Sagaing Region; Magway, Minbu, Naphe, Salin and Chauk from Magway Region.

SMOs were interviewed face-to-face with structured questionnaires regarding their personal data and job-related factors. Personal data involved demographic variables, training, distance between station hospitals (SH) and township hospitals (TH), transportation, situation, number of villages and population they served. Job-related factors of SMOs were confidentially kept. Data were entered, stored and managed by Epi-data. Data cleaning and consistency was done.

**RESULTS**

A total 32 SMOs participated in the study. Of which, majority (65.63%) were males and 34.37% were females. While 83.4% had graduated from Mandalay Medical University, 15.6% from Yangon Medical University. Mean age of SMOs was 36±2.88 years with the range from 29 to 41 years. Mean year of service was 7.06±1.83 with the range from 2 to 9 years.

On the average, each SMO had served about 13 villages (range: 13 to 68 villages) and 27764 population (range: 6424 to 53,000). Mean distance from station hospital to township hospital was 12.66 miles with the range of 5 to 27 miles (Table 1).

Table 1. Locality of station hospital

Characteristics	No.	%
Locality of station hospital		
Plain region	28	87.8
Hilly region	4	12.2
Transportation to station hospital		
Available	14	43.75
Not available	18	56.25
Public transportation from SH to TH		
Accessible	28	87.75
Inaccessible	4	12.25

SH-Station hospital  
TH-Township hospital

As for outlook toward their assigned job, perception, satisfaction, attitude and motivation toward their present task were considered (Table 2).

Table 2. Situation of job-related factors (n=32)

Outlook	Good	Poor
	No. (%)	No. (%)
Perception	18 (56.25)	14 (43.75)
Satisfaction	22 (68.75)	10 (31.25)
Attitude	18 (56.25)	14 (43.75)
Motivation	25 (78.25)	7 (21.75)

## DISCUSSION

Total Thirty-two SMOs from three divisions involved in the study, and most of them were males and in the middle-age group who have been served for 7 years on the average. The majority were graduated from Mandalay Medical University. They had served at least 13 villages with population coverage of about 28,000. They had to travel about 12 miles to reach the township hospital from their respective station hospital, where the majority of station hospital (87.75%) were located in plain region. More or less, there was not much difference for transportation and no difficulty for travelling to the respective township.

The SMOs from the study areas had positive outlooks regarding their present jobs. Similar situation were found among the rural doctors working for public clinics or hospital in Japan that they were satisfied with their work and life condition<sup>3</sup> and among health assistants in Myanmar.<sup>4</sup>

This study reveals that the SMOs from study areas had good attention in their present jobs. Though the SMOs in this study have positive outlook on their jobs, there is still need to explore details about the factors influencing the intension to stay in service or quit from the service, which will be helpful information in health Manpower Resource evaluation.

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## Knowledge on Reproductive Health and Reproductive Health Problems of Unmarried Women (25-49 years) in Three Selected Townships, Mandalay Region

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A community-based, cross-sectional descriptive study was conducted with the objective of assessing the existing reproductive health status of unmarried (24-49 years of age) women in three townships of Mandalay Region, PyinOoLwin, Amarapura and Singaing, from August 2008 to July 2009. Randomly selected 600 unmarried women were interviewed using the structured questionnaires. Main age group was 24-30 years (58.2%). One-third had primary education. They mentioned reproductive health problems like menstrual pain (57%) and irregularity (45.2%), pre-menopausal and post-menopausal symptoms (59.7%), lump in breast (47.4%), and uterine and cervical cancers (46.3%). Menstrual problems were reported at 29%. Most of them (94.2%) knew menarche, by physical appearance and menstruation. Approximately half of the study population knew at least three contraceptive methods. Urban (65%) and rural (62%) women responded that women can get pregnancy after single sexual exposure. Majority (94.5%) had heard of sexually transmitted diseases; HIV/AIDS (79.7%) and hepatitis B infection (8.5%). The methods of prevention were described as condom use (64%) and avoidance of sexual relationship with multiple sex partners especially with commercial sex workers (20.2%). Participants mentioned that times of voluntary HIV testing were before married (61.2%), before pregnancy (17.5%) and after performing risk behaviors (10.7%). Main sources of information concerning reproductive health were found to be television and video (63.7%) and newspaper/journal/magazines (31.7%). Health problem related to reproductive health was not identified.

### INTRODUCTION

Reproductive health is a universal concern, but is of special importance for women particularly during the reproductive years. Although most reproductive health problems arise during these productive years, in old age general health continues to reflect earlier reproductive life events.<sup>1</sup>

However, there is a cumulative effect across the life course events at each phase having important implications for future well-being. Failure to deal with reproductive health problems at any stage in life sets the scene for later health and developmental problems. Programmes dealing with various components of reproductive health exist in

some form almost everywhere. In general, such programmes exclusively targeted women, taking little account of the social, cultural and intimate realities of their reproductive lives and decision-making powers. They tended to serve only married people, excluding, in particular, young people and unmarried women.<sup>2, 3 & 4</sup>

The nuptiality pattern in Myanmar has been changing, the proportion of never-married has increased over the years at all ages for both sexes<sup>5</sup> but there was no research for unmarried women of age 24-49 years in Myanmar. Proportion of never-married among female was as high as 15% in age group 40-44 and 12% in 45-49. The percentage of the never-married women

aged 15-49 is 45.4 percent in 2001.<sup>6</sup> A comprehensive health promotion and disease prevention programme is offered to all women at or below 64 years of age to cater for their changing needs in different life stages from late adolescence/young adulthood to middle-age and those associated with role changes. Information concerning reproductive health is needed to know not only married women but also adolescents and unmarried women.

Therefore, this study focused on filling the knowledge and health problems gap concerning reproductive health in unmarried women who are increasing number in general population. It will also provide information on context of knowledge and health problems documented by survey such as knowledge on contraceptive methods, reproductive tract infections, HIV/AIDS, etc. Also information needed to develop programme will be collected, including an understanding of unmarried women's knowledge and their express needs.

## MATERIALS AND METHODS

A community-based cross-sectional descriptive study with face-to-face interview using pre-tested questionnaire was conducted among unmarried (never-married) women aged 24-49 years from PyinOoLwin, Amara-pura and Singaing townships, Mandalay Region. One hundred respondents from urban and one hundred from rural area of each township were selected randomly. Analysis was done by using SPSS software.

### *Ethical consideration*

The purpose and confidentiality of the study were explained to each person who participated in the study and informed consent was obtained.

## RESULTS

### *Scio-demographic characteristics of the respondents*

Most (58.2%) of respondents were 24-30 years old (Table 1). About 30.7% had attained at least primary level education;

own business (45.8%) and laborer (31.3%) because in these townships there were many domestic weaving business and own guardians. Head of family was found as father at 65%.

Table 1. Socio-demographic characteristics of the respondents

Variable	No.	%
<i>Townships</i>		
Amarapura	200	33.3
Singaing	200	33.3
PyinOolwin	200	33.3
<i>Residence</i>		
Urban	300	50
Rural	300	50
<i>Age groups (in years)</i>		
24 - 30	349	58.2
31 - 37	121	20.2
38 - 44	67	11.1
45 - 49	63	10.5
<i>Education</i>		
Primary school	184	30.7
Middle school	176	29.3
High school	150	25
University	20	3.3
Graduate	70	11.7
<i>Occupation</i>		
Dependent	99	16.5
Labouror	188	31.3
Bussiness owner	275	45.8
Government staff	38	6.4
<i>Head of family</i>		
Father	390	65
Mother	112	18.7
Sibling	26	4.3
Relatives	17	2.8
Self	55	9.2
<i>Main earner</i>		
Father	335	55.8
Mother	82	13.7
Sibling	70	11.7
Relatives	14	2.3
Self	99	16.5

### *Knowledge on reproductive physiology*

The respondents were asked to assess reproductive physiology of menarche, age of first menstruation, information regarding menstruation, source of information, health problems of menstruation and when they occurred. Most of the study women from urban (94.7%) and rural (93.7%) knew menarche. Among 600 unmarried women 149(24.8%) knew axillary hair, 508(84.7%) knew breast enlargement, 80(13.3%) knew pubic hair, 171(28.5%) knew acne, 197(32.8%) knew emotion/ shame as signs and symptoms of menarche as shown in Table 2.



Table 2. Knowledge on menarche and menstruation

Knowledge on menarche and menstruation	Responses (%)	
	Yes	No
<i>Do you know menarche?</i>		
Urban	94.7	5.3
Rural	93.7	6.3
<i>Sign and symptoms of menarche?</i>		
Axillary hair	24.8	75.2
Breast enlargement	84.7	15.3
Pubic hair	13.3	86.5
Acne	28.5	71.5
Emotion/shame	32.8	67.2
<i>Have you heard menstruation before?</i>		
Urban	84.3	15.7
Rural	85.0	15.0

Mean age of first menstruation was 14.9 years (SD±1.822). Majority (84.7%) had heard about menstruation before menarche. Their information perceived from mothers (46.8%), friends or neighbors (29.8%) and family members (36.2%).

Table 3. Knowledge on menstrual problems

Knowledge on menstrual problems	Responses (%)	
	Yes	No
<i>Do you know health problems relating menstruation?</i>		
Urban	97.3	2.7
Rural	98.0	2.0
<i>If yes, what are they?</i>		
Severe pain in low back and abdomen	95.5	4.5
Irregular menstruation	23.8	76.2
Prolong menstruation	12.5	87.5
Short period	11.0	89.0
Amenorrhea	10.8	89.2
Skin disorder and other	10.3	89.7
<i>Have you suffered above symptoms?</i>		
Urban	84.0	16.0
Rural	84.7	15.3
<i>If yes which one?</i>		
Low back and abdominal pain	76.3	23.7
Irregular menstruation	6.2	93.8
Prolong menstruation	2.5	97.5
Short period	3.2	96.8
Amenorrhea	2.8	97.2
<i>When do you suffer?</i>		
During period	52.8	47.2
Before period	42.7	57.3
After period	1.8	98.2
Irregular	0.8	99.2

### Menstrual problems

Table 3 shows that most of the respondents 586(97.7%) knew health problems relating menstruation which were severe pain in low back and abdomen (95.5%), irregular menstruation (23.8%), prolong menstruation (12.5%), short period (11%) and amenorrhea (10.8%). Of 600 respondents, more than

half (52.8%) suffered the symptoms during menstrual period. These symptoms were low back and abdominal pain (76.3%), irregular menstruation (6.2%), prolong menstruation (2.5%), short period (3.2%), amenorrhea (2.8%) and skin disorder and other (10.3%).

Regarding contraceptive methods, the majority of respondents (74.2%) claimed that they knew what contraceptives were and 71.2% knew pills, 69.5% injectables and IUDs at 34%, respectively. Most of the respondents did not know other methods like withdraw method, spermicidal and fertile period (Calendar method).

Respondents knew one to three contraceptive methods at 48.3% and more than three methods at 25.8%. Female sterilization at 45.8% and male sterilization at 14.8% were described as permanent methods. Majority (63.5%) knew that single sexual exposure can be pregnant.

Table 4. Knowledge on RTI/STI

Questions answered by participants (Yes)	Responses (%)	
	Urban	Rural
Do you know reproductive tract infection (RTI)?	64.0	71.0
Do you know sexually transmitted infection (STI)?	94.3	94.0
Are they different between RTI and STI?	35.3	41.0
Can these transmit from one person to another?	89.0	59.0
Low abdominal pain RTI?	7.3	2.7
Can RTI be prevented?	80.3	57.0
Methods of prevention; condom use	74.3	53.7
<i>Types of diseases transmitted by sexual contact</i>		
Syphilis	37.0	18.0
Gonorrhoea	3.3	2.0
Warts	3.3	0.7
HIV/AIDS	96.3	63.0
Hepatitis	12.3	4.7

### Knowledge on STIs/RTIs

Table 4 shows knowledge concerning RTI/STI. Urban unmarried women had more knowledge than rural women. Unmarried women had heard of reproductive tract infections RTI (67.5%) and sexually transmitted infections STI (94.5%). The respondents were also well informed about STI which can be transmitted (urban 89% vs. rural 59%). Unmarried women had heard about transmission from one person to

other by sexual contact at 74%. They had knowledge on sexually transmitted diseases like HIV/AIDS (79.7%), syphilis (27.5%), gonorrhoea (2.7%), venereal warts (2%) and hepatitis B (8.5%). Half of the respondents did not know symptoms of RTI in female. Unmarried women (412, 68.7%) answered STI can be prevented and mentioned methods of prevention as use condom (64%), faithful to spouse (13.3%), and avoidance of extra sex (20.2%). The information had come from health staff (21.7%), friends and neighbors (20.3%), voluntary health workers (1%), field survey staff (0.3%), health talks (23.7%), newspaper/journal/magazine (23.3%) and radio, TV, video (48.3%).

Table 5. Knowledge on reproductive health problems

Have you heard following reproductive health problems?	Yes response (%)	
	Urban	Rural
Severe low-back pain during menstruation	64.0	71.0
Irregular menstruation	51.7	38.7
Lump in breast	55.7	39.7
Lump in abdomen	20.0	16.0
Arthritis/ joint disorders	28.3	35.7
Pre- and post-menstrual symptoms	56.7	62.7
Cancer	52.7	40.0

### *Reproductive health problems*

Table 5 reveals unmarried women had heard reproductive health problems among which severe low-back pain during menstruation, arthritis/joint disorders and pre- and post-menstrual symptoms were more mentioned by rural unmarried women.

## **DISCUSSION**

The majority of respondents knew menarche, physical changes of menarche and menstruation. Menstruation and its disorders are not yet recognized as significant reproductive health morbidity. Overall knowledge regarding reproductive health seemed satisfactory in both urban and rural unmarried women. In present study, main compliant and presenting symptom was pain in low back and abdomen during menstruation. Similar findings were found in Nepal study.<sup>7</sup>

### *Knowledge on contraceptive methods*

In the present study, 72.4% of unmarried women knew different contraceptive methods. Among them more than 90% mentioned daily pills, secondly injection three month depot and thirdly Intrauterine Contraceptive Device (IUD). Sixty-five percent of participant knew one to three contraceptive methods.

According to a study in China by UNFPA during 2003, knowledge of any contraceptive method was 58.2% among never-married women and 99.4% among married women. Modern method knowledge, particularly reversible methods, was relatively low among young unmarried women, especially those residing in the western region. Many young women interviewed in the survey had low knowledge of condom. Only 9% of the never-married women had knowledge of 5 or more methods. Mean number of methods known was 1.7 among never-married women, 3.7 and 3.3 among married women and their husbands, respectively.<sup>8</sup> There was five-fold increase in reproductive health awareness among never-married females, a group that has traditionally been neglected by the family planning programme in China, although their awareness was still considerably less than that of their married counterparts.<sup>9</sup>

In the study on 1000 currently married women carried out with HO/HRP funding in rural area of Taikkyi Township, Yangon Region, Myanmar, 99% had heard of contraception, the most commonly known method was pills followed by injection depot.<sup>10</sup>

### *Knowledge on RTI/STI*

Participants had heard various types of STI in which mostly known infection was HIV/AIDS but most of them did not know hepatitis B can be transmitted by sexual contact. The majority of participants had satisfactory knowledge about routes of transmission of HIV/AIDS and STD. This agrees with results of other studies including the Tanzanian demographic and health survey which showed that 80% of partici-

pants knew that the diseases can be transmitted by sexual contact.<sup>11, 12</sup> Our results do not support a study on teachers in South Africa which indicated very poor general knowledge about transmission of HIV<sup>13</sup> and a study in Calcutta in which only 16.2% of teachers had clear knowledge about HIV transmission and prevention.<sup>14</sup>

#### *Reproductive health problems*

The majority of women had heard of menstrual pain and irregularity, premenopausal and post-menopausal symptoms (tingling, numbness, hot flash, etc.), lump in the breast and uterine and cervical cancer. Among those health problems, they suffered mainly menstrual pain and it did not need specific treatment.

#### *Conclusion*

Urban never-married women knew more methods of contraception, types of STI including hepatitis B infection and symptoms of RTI than rural women. Graduate women had more knowledge on HIV/AIDS including causal agent, route of transmission, methods of prevention, who prone to get infection and time for HIV test. Therefore, health education programme concerning reproductive should focus more on rural and low educated groups. Major source of information was media; it was also important message for IEC campaign. Health problem related to reproductive health was not identified during study period.

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**Learning Styles of Medical Students of Different Grades  
of University of Medicine (Magway)**

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Students receive information in a variety of modes. Knowledge on the ways they learn and process information will help develop effective instructional strategies and methods which make effective learning. To identify the learning styles of medical students of different grades of University of Medicine (Magway), the VARK questionnaires were administered to a total of 559 medical students; 182 first-year medical students, 196 third-year medical students and 181 final part II medical students in March 2010. Students from all 3 grades utilized multimodal learning style more commonly than a single mode (n=100, 18%). Among them, bimodal learning style was used by most of the students (bimodal - n=189, 41.2%; trimodal - n=147, 32%; all modes - n=123, 26.8%). Reading/writing learning style was found to be the most commonly used modality, followed by auditory learning style, kinesthetic learning style and then visual learning style (R=38, A=32, K=22, V=8) among unimodal preferences. In students with multimodal learning style, reading/writing learning style was found to be commonly used modality followed by auditory learning style, visual learning style and kinesthetic learning style in different combinations. Auditory and visual combination (AV) was the least commonly used mode in both first-year students and third-year students whereas more preference for AV was seen in final part II students. First-year students preferred reading/writing mode to other modes. Third-year students showed equal preference for reading/writing mode and auditory mode whereas final part II students preferred auditory mode to other modes. Learning style changes with age. Changes from reading/writing and kinesthetic modes in first-year students to auditory and visual (AV) in final part II students were seen.

**INTRODUCTION**

People differ consistently from each other in their preferences (e.g., emotional, environmental) for certain ways of information processing. Learning style is the way students begin to concentrate on, process, internalize, and remember new and difficult academic information.<sup>1</sup> There are four factors that significantly differ between groups and among individuals for learning style: global versus analytic processing styles, age, gender, and high- versus low-academic achievements.<sup>2</sup> There are many thoughts and theories about individual

learning styles. Learning strengths may also be classified as Learning Style Inventory (modality): Visual, Auditory, Kinesthetic and Tactile, Hemispheric Dominance, Differences Between Left and Right Hemisphere, Hemispheric Dominance Inventory; Kolb's Learning Styles model (David Kolb's perception vs. processing): Learning Style Inventory (active/reflective, sensing/intuitive, visual/verbal, sequential/global); Myers Briggs Type Indicator Instrument: The Use of Learning Style Innovations to Improve Retention; Personality Type Summary: Descriptions of four personality types, Center of Psychology Resources; The

Multiple Intelligence Inventory: Using Multiple Intelligences, Gardner's Seven Types of Intelligence, Seven Styles of Learning or Situational and Activist, Reflector, Theorist or Pragmatist.<sup>3, 4</sup> Fleming's VARK model was one of the most common and widely-used categorizations of the various types of learning styles.<sup>5, 6</sup>

Visual learners learn through seeing. They learn best from visual displays including diagrams, illustrated text books, overhead transparencies, videos, flipcharts and handouts. Visual learners often prefer to take detailed notes to absorb the information. Auditory learners learn through listening. These learners often benefit from reading text aloud and using a tape recorder. Read-write learners prefer printed words and texts, lists, glossaries, textbooks, lecture notes, or handouts. Tactile/Kinesthetics learners learn through moving, doing and touching.<sup>7</sup>

It has been shown that there are significantly higher learning gains for college students when instructional strategies/resources compliment student learning styles.<sup>8</sup> To provide all students an equal opportunity to succeed, teachers have to implement various teaching methods in order to captivate each student's attention. The present study is aimed to identify and compare the learning styles of medical students of different grades of University of Medicine (Magway).

## MATERIALS AND METHODS

To determine the preferred mode(s) of learning, English version of the VARK questionnaires<sup>9</sup> for young were administered at the end of the first semester (March, 2010) to 182 first-year medical students, 196 third-year medical students and 181 final part II medical students of University of Medicine (Magway). This study was performed at the Department of Microbiology, University of Medicine (Magway) in March 2010.

### *Administering the questionnaire*

When instructing the students to fill in the questionnaire they were told to make a selection (a, b, c or d) for each question, but they could omit a question or they could choose more than one option if they want to. Information on the meaning of words in the questionnaire and additional contextual or situational information will not be given before they choose their answers as it may bias responses to the questions. They could choose more than one response if they think the context is not clear. Before they complete the questionnaire they will be informed that the results indicate their preferences but are not necessarily their strengths.

## RESULTS

Among the first-year medical students, 15.4% preferred unimodal learning style and 84.6% preferred multimodal learning style. The third-year medical students who used unimodal learning style and multimodal learning style were 15.8% and 84.2%, respectively. Of final part II medical students, unimodal learning style was 22.7% and multimodal learning style was 77.3% in percentage distribution.

Of the different grades of medical students who preferred unimodal learning style, the first-year medical students preferred reading/writing (R-42.9%) and kinesthetics mode (K-28.6%). The third-year medical students and final part II medical students preferred reading/writing (R-41.9% and 31.7%) and auditory (A-41.9% and 36.6%) mode of unimodal learning style (Table 1).

Table 1. Percentage distribution of unimodal learning style of medical students of different grades

Grades	Visual No. (%)	Auditory No. (%)	Reading/ Writing No. (%)	Kinesthetics No. (%)	Total
First-year	4(14.3)	4(14.3)	12(42.9)	8(28.6)	28
Third-year	0	13(41.9)	13(41.9)	5(16.1)	31
Final part II	4(9.8)	15(36.6)	13(31.7)	9(21.9)	41
Total	8(8)	32(32)	38(38)	22(22)	100

For multimodal learning style, first-year medical students preferred bimodal learning style to trimodal and quadrimodal learning styles (48% vs. 33.1% and 18.8%). Final part II students also preferred bimodal learning style to trimodal and quadrimodal learning styles (45% vs. 27.9% and 27.1%). Among the third-year medical students, bimodal, trimodal and quadrimodal learning styles were 31.5%, 34.5% and 33.9%, respectively, in percentage distribution (Table 2).

Table 2. Percentage distribution of multimodal learning style of medical students of different grades

Grades	Bimodal No. (%)	Trimodal No. (%)	Quadrimodal No. (%)	Total
First-year	74(48.0)	51(33.1)	29(18.8)	154
Third-year	52(31.5)	57(34.5)	56(33.9)	165
Final part II	63(45.0)	39(27.9)	38(27.1)	140
Total	189(41.2)	147(32)	123(26.8)	459

Table 3. Percentage distribution of bimodal learning style of medical students of different grades

Grades	VK No. (%)	VA No. (%)	VR No. (%)	AK No. (%)	RK No. (%)	AR No. (%)	Total No.
First-year	9 (12.2)	6 (8.1)	13 (17.6)	9 (12.2)	14 (18.9)	23 (31)	74
Third-year	4 (7.7)	3 (5.8)	12 (23)	6 (11.5)	8 (15.4)	19 (36.5)	52
Final part II	1 (1.6)	13 (20.6)	7 (11.1)	7 (11.1)	7 (11.1)	28 (44.4)	63
Total	14 (7.4)	22 (11.6)	32 (16.9)	22 (11.6)	29 (15.3)	70 (37)	189

VK = Visual & kinesthetics mode  
 VA = Visual & auditory mode  
 VR = Visual & reading/writing mode  
 AK = Auditory & kinesthetics mode  
 RK = Reading/writing & kinesthetics mode  
 AR = Auditory & reading/writing mode

Table 3 presents percentage distribution of bimodal learning style among different grades of medical students. Auditory and reading/writing mode (AR) was found to be the most commonly used combination used by all students from the three grades (first-year 31%, third-year 36.5% and final part II 44.4%). First-year medical students utilized more reading/writing and kinesthetics mode (RK-18.9%) and visual and reading/writing mode, (VR-17.6%) than visual and kinesthetics (VK-12.2%), auditory and kines-

thetics (AK-12.2%) and visual and auditory mode (VA-8.1%). The third-year medical students used reading/writing and kinesthetics mode (RK-15.4%) less frequently than visual and reading/writing combination (VR-23%). Final part II students preferred visual and auditory mode (VA-20.6%) to other bimodal learning styles.

Table 4. Percentage distribution of trimodal learning style of medical students of different grades

Grades	ARK No. (%)	VAK No. (%)	VAR No. (%)	VRK No. (%)	Total
First-year	14(27.4)	5(9.8)	18(35.3)	14(27.5)	51
Third-year	11(19.3)	11(19.3)	26(45.6)	9(15.8)	57
Final part II	16(41.0)	0	17(43.5)	6(15.4)	39
Total	41(27.9)	16(10.8)	61(41.5)	29(19.7)	147

ARK = Auditory, reading/writing & kinesthetics mode  
 VAK = Visual, auditory & kinesthetics mode  
 VAR = Visual, auditory & reading/writing mode  
 VRK = Visual, reading/writing & kinesthetics mode

Medical students of all grades preferred visual, auditory and reading/writing mode (VAR-35.3%, 45.6% and 43.5%) to other trimodal learning styles. First-year students and final part II students preferred visual, reading/writing and kinesthetics mode (VRK-27.5% and 15.4%), and auditory, reading/writing and kinesthetics mode (ARK-27.4% and 41%) to visual, auditory and kinesthetics mode (VAK-9.8%, 0%). But the third-year medical students showed preference on visual, auditory and kinesthetics mode (VAK-19.3%) and auditory, reading/writing and kinesthetics mode (ARK-19.3%) to visual, reading/writing and kinesthetics mode (VRK-15.8%) (Table 4).

## DISCUSSION

Current increased student intakes cause entry of medical students from diverse backgrounds. There is also a growing emphasis on developing higher order cognitive skills of university science students, ie., the capacity to solve problems, to analyse and classify data, to gather evidence about solutions and to apply and test theories. To support learning of students to cope with as many new terms and area, expanding

scientific knowledge over emphasis on rote learnt content and terminology forces students into surface learning approaches. Opportunities to develop understanding of concepts and learning process skills, to practice them need to be given to learners in the context of the subject matter domains where they will have to use them.

Students receive information in a variety of modes because they are different with unique strength, talent, weakness, learning styles and preferences. Knowledge on the ways they learn and process information will help develop effective instructional strategies and methods which make effective learning. Students learn more in a manner compatible with their own learning preference. Blending of different learning methods is more effective than using one method only.<sup>10</sup>

Visual learners remember things best by things written down in a handout, text or on the overhead such as drawing, maps, pictures, diagrams, demonstrations, graph, chart, flow diagrams, display, computer graphics, cartoons and film will benefit visual learners who prefer to see. Visual learners as well as kinesthetic learners will value to-do lists, assignment logs, and written notes.<sup>11</sup>

Replacing words with symbols or initials, translating concepts into pictures and diagrams, underline or highlighting notes or textbooks with different colors, practicing turning visuals back into words, and making flashcards of key information with words, symbols, and diagrams should be used for the visual learning style.

Auditory learners tend to benefit most from traditional teaching techniques, lecture; also gain benefit from reading aloud, interviewing, debating, participating on a panel and giving oral reports. Discussion, questions, answers, games, using tapes and audio enhance the auditory learner's classroom experience.<sup>12</sup>

Learning strategies for the aural learning style include attending lectures and tutorials, discussing topics with teachers and other

students, putting summarized notes on tape and listening to them, joining a study group, and talking out loud.<sup>13</sup>

Reading/writing learners learn best by reading printed materials, writing out important information again and again, reading notes silently, organizing any diagrams into statements, rewriting the ideas and principles in other words, making flashcards of words and concepts that need to be memorized and reading important points or outlines of the lecture material.<sup>13</sup>

Kinesthetic learners prefer role play, practical class, drama, things to build, drawing, playing board games, making dioramas, making models, movement, sports and physical games, tactile experience or hands-on experience, field trips, visiting museums, studying with others, setting up experiments, using memory games, using flash cards to memorize promote deep learning. They will do best in answering definitions, fill-ins and multiple choice.<sup>14, 15</sup>

Learning strategies for the kinesthetic/tactile learning style include read out loud from textbook and notes, copy key points onto large writing surfaces (i.e. chalkboard or easel board), copy key points using word processing software, listen to audiotapes of notes while exercising, take in information through field trips, laboratories, trial and error, exhibits, collections, and hands-on examples summary, recall experiments and role-play, and use pictures and photographs that illustrate an idea.<sup>13</sup>

In this study, learning styles among 559 medical students of first year, third year and final part II were multimodal learning style (82.1%) and unimodal learning style (17.9%). Percentage utilization of multimodal learning style was greater than that of unimodal learning style among all three grades. (first-year 84.6% vs. 15.4%; third-year 84.2% vs. 15.8% and final part II medical students 77.3% vs. 22.7%).

Students who preferred unimodal learning styles utilize reading/writing, auditory, kinesthetics and visual modes in decreasing frequency (R-38%, A-32%, K-22%, V-8%).

First-year students preferred reading and kinesthetics (R-42.9%, K-28.6%) to other modes. When compared to it third-year and final part II students showed preference on auditory and reading modes (A-41.9%; R-41.9% and A-36.6%; R-31.7%), respectively. There was no visual learners in third-year students and small numbers in final part II students.

One hundred and eighty-nine students (41.2%) of multimodal learning style prefer bimodal modes followed by trimodal (32%) and then quadrimodal learning styles (27%). First-year medical students and final part II students preferred bimodal learning style to trimodal and quadrimodal learning styles (first-year 48% vs. 33.1% and 18.8%) (final part II 45% vs. 27.9% and 27.1%). In contrast, equal distribution of bimodal, trimodal and quadrimodal learning styles were observed among the third-year medical students (31.5%, 34.5% and 33.9% in percentage distribution, respectively).

Although different combinations of reading, visual, auditory and kinesthetics modes were seen in different grades, most frequently utilizing combinations were auditory reading (AR) (first-year 31%, third-year 36.5%, final part II 44.4%), visual reading (VR) (first-year 17.6%, third-year 23%), kinesthetic reading (KR) (first-year 18.9%, third-year 15.4%, final part II 11.1%) and visual auditory reading (VAR) modes (first-year 35.3%, third-year 45.6%, final part II 43.5%). Reading/writing and kinesthetics modes of learning predominate in the first-year students who prefer single mode and two learning modes.

Compared to first-year students (VK=12.2%) and third-year students (7.7%), final part II students (1.6%) were weak in VK combination learning style. Population of both first-year students (VA=8.1%) and third-year students (VA=5.8%) who utilized visual and auditory combination (VA) were small in bimodal modes. However, more preference for VA (20.6%) was seen in final part II students. Learning styles change with age; many people's styles alter as they grow

older; for many people, auditory and visual perceptual elements strengthen with age.<sup>2</sup> Our findings were compatible showing changes from reading/writing and kinesthetics modes in first-year students to auditory and visual (AV) in final part II students.

Students of University of Medicine (Magway) represent a diversity of age, experience, culture, ethnicity and learning preference. There will be problems when students with one mode of learning exposed to instruction that required other modes of learning.

Therefore, it is important to provide a variety of learning to meet for the educational needs of all students to motivate and improve performance of them and also make students to adapt to other modes of learning in addition to preferred mode. The techniques that engage students captivate students' attention and interest in the material stimulate students' mental activity and the techniques are more feasible, new and exciting make effective for students to learn. Methods that intentionally combine information processing across learning styles should be used in order to accommodate every student's unique learning style and for supporting academic success for more students.

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## Knowledge and Practice of Safety Measures on Agricultural Pesticide Utilization among Farm Workers in Kyauktan Township

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Pesticides play an important role in prevention and control of pests and diseases of plants. The use of agricultural pesticide is common in maintaining and increasing crop yields. Pesticide poisoning is one of the specific occupational health problems among agricultural workers. In order to prevent pesticide toxicity in work place, safety precautions would be needed. The specific objectives of this study were to explore the awareness and practice of safety measures on pesticide utilization among farm workers and to find out the relationship between knowledge and practice of pesticide utilization of farm workers. A total of 141 farm workers from the three villages of Kyauktan Township, Yangon Region were involved in a cross-sectional descriptive study. Most of the respondents (70.2%) knew about wearing masks for protective measures and very few knew to wear apron and protective clothes. There was statistically significant association between educational status of the respondents and level of knowledge of safety measures on pesticide utilization in this study ( $p < 0.05$ ). Statistically significant association between level of knowledge and level of practice of farm workers ( $p < 0.05$ ) was also found. From an overall of the findings it is apparent that farm workers did not have adequate knowledge and proper practice for safe precaution against chemical pesticides. Thus, health education programmes to promote personal protective equipment are highly essential.

### INTRODUCTION

Pesticides play an important role in prevention and control of pests and diseases of plants. Agricultural pesticides are commonly used to maintain and increase crop yields, while public health pesticides are applied to prevent vector-borne diseases. Both types are also used in homes and gardens. A pesticide is defined as a substance or mixture of substances intended for preventing, destroying or controlling any pest including vectors of human or animal disease, unwanted species of plants or animals causing harm during or otherwise interfering with the production, processing, storage, transport or marketing of food Agricultural commodities, wood and wood products or animal food-stuff or which may

be administered to animals for the control of insects, arachnids or other pests in or their bodies.<sup>1</sup> Pesticide poisoning is one of the agricultural hazards. Myanmar is an agricultural country. Farm workers in Myanmar have to deal with pesticides and they can have health hazards due to nature of their work. Accidental exposure or over exposure to pesticides can have serious health implication. So, safety utilization of pesticides is very important to reduce harmful effects on agricultural workers. According to the World Health Organization<sup>2</sup>, poisoning can be prevented if pesticides are used safely and proper precautions are taken.

A local study on acute poisoning conducted from October 2002 to September 2003 showed that 24.4% of acute poisoning was

due to pesticide poisoning.<sup>3</sup> Health hazards of pesticides can be reduced by proper understanding of safety measures of pesticide utilization and practice of using personal protective equipments (PPEs).

This study is expected to reveal the knowledge and practice of farm workers about safety measures of pesticides utilization. The objectives of the study were to assess the knowledge on health hazards and safety measures of pesticide utilization among farm workers; to determine the practice of using PPE and following the safety measures of pesticide among farm workers; and to find out the relationship between knowledge and practice of pesticide usage of farm workers.

## MATERIALS AND METHODS

A cross-sectional descriptive study was undertaken among farm workers including farmers and gardeners in three villages (Pantaw village, Ywarthitkalay village, and Kanpyaung village) of Kyauktan Township, Yangon Region. Sample size was calculated by using the sample size calculation formula:

$$n = z^2 p q / d^2$$

n = Minimum required sample size  
z = Reliability coefficient (1.96)  
p = Proportion of farm workers who followed the correct safety precautions after handling pesticide 77%<sup>4</sup>  
q = 1 - p (23%)  
d = Absolute precision (0.07)  
f = Non-response rate (5%)  
 $n = (1.96)^2 \times 0.77 \times 0.23 / (0.07)^2$   
= 138.84  
= n (1/1-f)  
= 138.84 (1/1-0.05)  
= 146.14

Hence, a total of 150 farm workers were planned to be included. Ten farms were selected from each village purposively. In each farm, five farmers who met the inclusion criteria (farm workers who actually work on the farm either as an owner or a worker; age 18 years and above; and sex, both male and female) were chosen

by using a multi-stage sample method (Fig. 1). The final sample size achieved was 141, with 46, 47 and 48 from Pantaw village, Ywarthitkalay village and Kanpyaung village, respectively. Face-to-face interview using pre-tested structured interview questionnaire was conducted. The purpose of the study was explained to the respondents and informed consents from all respondents were obtained before the interview. Each Interview was carried out in a place where there was privacy.

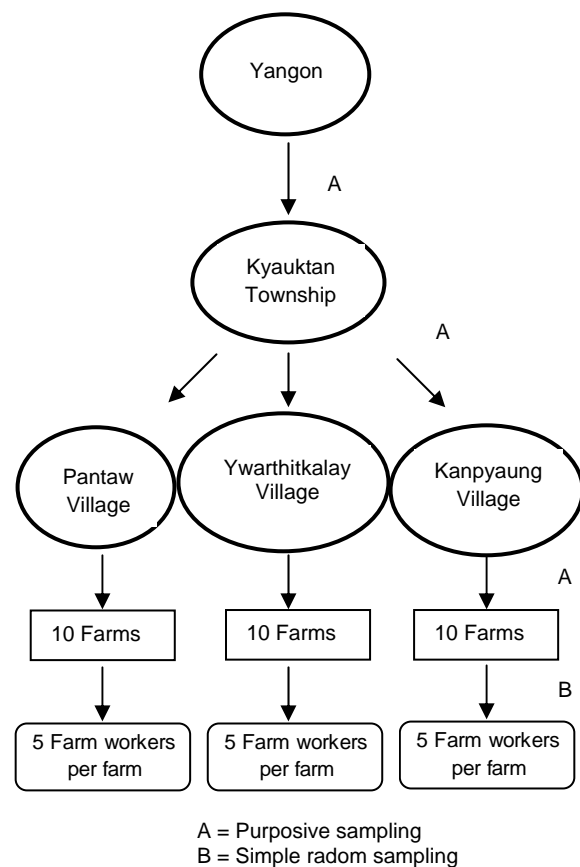


Fig. 1. Sampling method and procedure

At the end of each interview, the questionnaire was checked in order to see if there were errors and incompleteness in their answers. Coding in the questionnaire was checked and entered into the computer by using SPSS 16.0 software. After data entry had been completed, the data were cleaned up by looking at the total frequencies, frequency distribution. Data clarifying was also done by cross tabulation.

## RESULTS

Ages of respondents ranged from 18 years to 77 years, with mean age of 42.82; males are about twice of females; most have finished primary level of education (56%); most are farm owners (62.4%), followed by arm workers and garden owners; the largest number of respondents has been working for 6 to 30 years and only about 11% works for more than 30 years; and most of the respondents have less than ten years duration of pesticide use and very few have used pesticide from 20 years up to 35 years.

### *Knowledge of pesticides*

There are 9 main questions for scoring. They are knowledge of pesticide, harmful effects of pesticide, route of entry of pesticide, toxicity of pesticide, chemical residue, pesticide label, dangerous if mixed with food, disposing of pesticide and PPEs. In scoring, concerning with response to knowledge 'Yes' is given as score 1 and 'No' as 0. Level of knowledge is categorized as good and poor. Knowledge score below the mean is regarded as low knowledge and mean score and above are regarded as high knowledge. Out of total knowledge score allotted, 9, mean score is 5.8 with SD of 2.42, and the range is from 0 to 9. It is found that 60% has good knowledge.

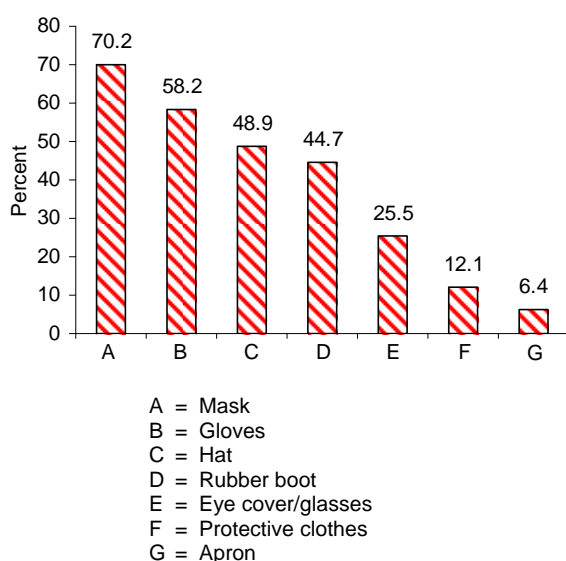


Fig. 2. Distribution of knowledge of PPE

Figure 2 shows that most of the respondents (70.2%) know about wearing mask for protective measures and a very few know they should wear aprons and protective clothes.

### *Practice of safety measures on pesticide*

Practice of safety measures on pesticide utilization among respondents is determined by storage, mixing, and safety measures during spraying pesticides, systematic disposal of pesticide containers, and application of spraying apparatus, using PPE and taking safety precaution after handling pesticides. Total practice score allotted is 27 and mean score of practice got by respondents is 14.0 with SD of 4.52. Range of practice score is from 2 to 23. Practice score below the mean is set as low practice and mean practice score and above are set as high practice. It is found that about 53.9% have high practice score and 46.1% have low practice score.

Regarding the use of PPE, only 38 respondents (27%) did not. Among PPE, hats, caps or other covers were most common in use (74.5%). Other correct protections such as wearing mask (73.6%), gloves (62.3%), rubber boots (50.9%) and goggles, and using face shield (23.6%) were also found. The participants who use aprons and protective clothes were rare, being 3.8% and 8.5%, respectively.

### *Association between background characteristics and knowledge level*

From Table 1, it can be noted that the respondents who were in the age group of 31 to 50 years had high knowledge (62.2%). Both genders had approximately the same level of high knowledge. Respondents of higher education level had higher knowledge level than those of low education level, and it was statistically significant ( $p < 0.05$ ) and the respondents who had longer duration of farm work were not higher than those who had shorter duration of farm work.

Table 1. Associations between background characteristics of study population and knowledge level (n=141)

Background characteristics	Knowledge level	
	Low No. (%)	High No. (%)
<i>Age group (years)</i>		
≤30	9(39.0)	14(61.0)
31-50	31(37.8)	51(62.2)
≥51	17(47.2)	19(52.8)
<i>Gender</i>		
Male	37(38.9)	58(61.1)
Female	20(43.5)	26(56.5)
<i>Education*</i>		
Low education	48(50.5)	47(49.5)
Median education	8(48.1)	25(51.9)
High education	1(7.7)	12(92.3)
<i>Occupation</i>		
Farm owner	38(43.2)	50(56.8)
Garden owner	5(27.8)	13(72.2)
Farm worker	14(40.0)	21(60.0)
<i>Duration of work (years)</i>		
≤5	10(41.7)	14(58.3)
6-15	17(33.3)	34(66.7)
16-30	21(41.2)	30(58.8)
>30	9(60.0)	6(40.0)

\*Statistically significant at p<0.05

Table 2. Associations between background characteristics of study population and practice level (n=141)

Background characteristics	Practice level	
	Low No. (%)	High No. (%)
<i>Age group (years)</i>		
≤30	8(34.8)	15(65.2)
31-50	41(50.0)	41(50.0)
≥51	16(44.4)	20(55.6)
<i>Gender</i>		
Male	44(46.3)	51(53.7)
Female	21(45.7)	25(54.3)
<i>Education</i>		
Low education	50(52.6)	45(47.4)
Median education	13(39.4)	20(60.6)
High education	2(15.4)	11(84.6)
<i>Occupation</i>		
Farm owner	41(46.6)	47(53.4)
Garden owner	5(27.8)	13(72.2)
Farm worker	19(54.3)	16(45.7)
<i>Duration (years)</i>		
≤ 5	13(54.2)	11(45.8)
6 -15	19(37.3)	32(62.7)
16-30	27(52.9)	24(47.1)
> 30	6(40.0)	9(60.0)

*Association between background characteristics and practice level*

From Table 2, it can be seen that the younger age group can have high practice (65.2%). Both genders had the same level of practice. About 84.6% of farm workers who had high education had high practice. Some farm owners had high practice level but

some did not. A person with a shorter duration of farm work can have high practice (62.7%).

Table 3. Association between knowledge level and practice level

Knowledge level	Practice level		Total
	Low No. (%)	High No. (%)	
Low	35(53.8)	22(46.2)	57(100)
High	30(28.9)	54(71.1)	84(100)
Total	65(40.4)	76 (59.6)	141(100)

*Association between knowledge level and practice level*

It is found that there is a statistically significant association between the levels of knowledge and practice (Table 3).

**DISCUSSION**

Regarding the knowledge about PPE, more than 90% of farm workers had knowledge about hat, mask, and gloves and nearly 70% knew about goggles or eye cover and boots and 17.5% mentioned aprons in a previous study.<sup>4</sup> In the study done at pesticide formulating plant, more than 90% of pesticide production workers had knowledge on mask, gloves and apron and about one-third of them had knowledge about boots and goggles.<sup>5</sup>

Farm workers of this study had less knowledge about PPE. The knowledge of protective clothing and aprons was not as common as masks and gloves because the latter were more easily accessible than the former ones. Besides, especially in rural areas, Myanmar housewives hardly wear aprons and the aprons even in the kitchens and the aprons intended for farmers are harder and thicker. So, farm workers were not keen on wearing aprons at work place as they were too uncomfortable to wear while working in the tropical climate. Special designs and materials should be used for making aprons as protective device.

The lack of knowledge on PPE is due to insufficient knowledge about the importance

of these accessories. The more knowledge farm workers have concerning the importance of PPE, the more protective measures they will take and the less they will be exposed to the danger caused by the use of pesticide.

In this study, the association between educational status and knowledge level of farm workers is found to be significant. Farm workers of high education level had more knowledge than those of low education level. The majority of the respondents had primary education. The respondents who had high school and university educational level had high knowledge 8.5% (12/141) and most of the respondents who had primary education level had low knowledge 34.0% (48/141). It is also noted that educational status and knowledge level of respondents relate to each other. Farm workers with high school education and the graduates possessed higher level of knowledge.

In this study, there was lack of association between age, sex, duration of work and occupation in both knowledge level and practice level. So, there is also no association between socio-demographic of farm workers and their practice. In a study, it was found that association between gender and knowledge of farm workers was significant.<sup>4</sup> There was association between educational status of farm workers and level of knowledge about safety measures on pesticide utilization.

In this study, there is no statistically significant association between occupation and practice level of the respondents. Owners as well as workers had same level of knowledge. In comparison with another study conducted in Hmawbi concerning pesticide formulation plant<sup>5</sup>, although the nature of work and characteristics of production workers (exposed workers) and farm workers in Hmawbi study are different, the state of knowledge about safety measures is not much different. This indicates that the pesticides handlers, whether the subject is either a production

worker or farm worker, are required to have the general knowledge of pesticides and knowledge on safety measures.

According to a cross-sectional study among farm workers in Hmawbi Township, the majority of respondents had more knowledge in safety measures than general knowledge of pesticides. But utilization of PPE among farm workers was not enough to have chemical safety for them. It was also found that association between knowledge and practice of farm workers was statistically significant.<sup>4</sup>

In this study, it was found that association between knowledge and practice of farm workers was statistically significant. Farm workers who had high knowledge applied the knowledge in practice. Lack of knowledge leads to insufficient practice thus it can be seen that the higher the knowledge level, the higher the practice level.

Concerning association between knowledge and practice and socio-demographic characteristics and association between knowledge and practice, educational status of farm workers helps in getting high knowledge on pesticides utilization. Higher level of education status may lead to higher level of knowledge and less chance of toxicity. So, it is essential to provide education and training concerning with pesticide utilization.

It is necessary for health personnel to educate farm workers concerning pesticide utilization and to promote their knowledge as well. This is because the ways of spreading knowledge about safety measures via media are not much effective especially the use of protective clothes or aprons. It will be more effective if health workers provide knowledge by explaining pros and cons.

Utilization of PPE among farm workers in this study is not enough to have chemical safety for them. Thus, further enhancing of education regarding PPE is suggested for reducing risks of pesticides among farm workers in the study area. To promote safety

practice of chemical pesticides, providing knowledge to attain behavioural changes of pesticide users is one of the important factors.

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## Seasonal Prevalence and Biting Patterns of Malaria Vectors in Hard-to-reach Area of PyinOoLwin Township

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In Myanmar, malaria is still recognized as one of the public health problems and usually malaria endemic areas are located in forested, rural, hilly or mountainous and hard-to-reach areas. The objective of this study was to determine the seasonal prevalence of malaria vectors and their biting patterns. Mosquitoes were collected with human baits (indoor and outdoor) and animal baited trap nets from October 2008 to July 2009 in Thayetpininn Village, PyinOoLwin Township, Mandalay Region. A total of 1814 *Anopheles* mosquitoes with 15 *Anopheles* species were collected. Primary vector, *Anopheles minimus*, was the predominant vector in premonsoon season (May). Most abundantly collected secondary vectors were *An. maculatus* in premonsoon (May), and both *An. annularis* and *An. philippinensis* in postmonsoon (October). The results of the biting patterns on human baits showed that *An. minimus* were early-night biters especially in the first quarter (6:00 pm - 9:00 pm) of night. The biting patterns of *An. maculatus*, *An. annularis* and *An. philippinensis* on human baits also showed them as early-night biters especially in the first quarter of night. *An. minimus*, *An. maculatus*, *An. annularis* and *An. philippinensis* preferred to feed on human baits outdoor than that of indoor. The results of this study are expected to be useful in personal protection against mosquitoes in this area.

## INTRODUCTION

In Myanmar, malaria is still recognized as one of the public health problems and has spread throughout the country. Malaria endemic areas are usually located in forested, rural, hilly or mountainous and hard-to-reach areas. Among the factors maintaining malaria endemicity in large parts of the country are climatic changes, migration of non-immune populations into malarious areas, ecological changes, existence of multi-drug resistant malaria parasite and appearance of insecticide-resistant vectors.<sup>1</sup> Another factor that has contributed strongly to maintaining the malaria disease is lack of knowledge of the biological behavior of the different species

of *Anopheles* involved.<sup>2</sup> The transmission of malaria disease depends mainly on the presence of optimal environmental conditions for the suitable *Anopheles* vectors. About 60% of all malaria cases in Myanmar are reported from the forest and forest fringe areas.<sup>3</sup>

Among the *Anopheles*, 37 species including *Anopheles dirus*, *An. minimus* (primary vectors) and many potential vectors *An. annularis*, *An. sudaicus*, *An. aconitus*, *An. maculatus*, *An. culicifacies* etc. have been found in Myanmar.<sup>4</sup> Many anopheline mosquitoes have differed in habits and habitats, so that control strategy will also differ. This knowledge is needed to accurately assess disease transmission patterns within each specific area, so as to



be able to implement anopheline control measures.<sup>2</sup> The objective of this study was to determine the seasonal prevalence of malaria vectors and their biting patterns.

## MATERIALS AND METHODS

### *Study area*

Thayetpininn Village, PyinOoLwin Township, Mandalay Region with 76 houses and 452 inhabitants was selected as study site. There is no health centre, no public transportation and market. The main occupations of villagers are agriculture, seeking forest products and high land cultivation.

### *Entomological studies*

The surveys were done in October 2008 (postmonsoon), February 2009 (cool dry season), April 2009 (hot-dry season), May 2009 (premonsoon) and July 2009 (monsoon). Four catching stations were chosen in the selected area. All catches were done in fixed stations throughout the study periods. Duration of each survey was 5 days.

### *Entomological sampling techniques*

Human baits (indoors and outdoors collection) were employed to catch anophelines landing on bare legs with test tube was conducted hourly starting from 18:00 hours to 06:00 hours of the next morning. Human baited and animal baited big bed net (11 ft x 11 ft x 6 ft) catches were also conducted with aspirator hourly from 18:00 hours to 06:00 hours of the next morning. The study was divided into four quarters of biting times. First quarter started from 18:00-19:00 to 20:00-21:00 hour, second quarter was from 21:00-22:00 to 23:00-24:00 hour, third quarter conducted from 24:00-01:00 to 02:00-03:00 hour and fourth quarter from 03:00- 04:00 to 05:00-06:00 hour.

### *Larval collection*

Larval surveys were carried out within 3 km radius of the study area. At the study site, the collection of larval was done by using standardized dipper.

### *Entomology variables*

Anopheline mosquitoes (adult & larva) were identified morphologically. The ovaries were dissected to determine the parous rates and vector incriminations by salivary gland dissection were conducted.<sup>5</sup>

### *Meteorological data*

Data were obtained from meteorological station of PyinOoLwin. The highest rainfall was 14.29 inches in October, 2008. The lowest and the highest relative humidity were 61% in April and 89% in July, 2009, respectively. The lowest temperature was 8°C in January, 2008 and the highest was 30°C in June, 2009, respectively. During the study period, the rain fall 3.55 inches was found in April and 5.86 inches was found in early May, 2009, respectively.

### *Ethical consideration*

Informed consent was obtained from all the participants. In addition, each was provided with antimalarial prophylaxis.

## RESULTS

The seasonal prevalence of 1814 *Anopheles* mosquitoes with 15 *Anopheles* species collected from October 2008 to July 2009 were determined as shown in Table 1.

A total of 460 *Anopheles* mosquitoes with 13 *Anopheles* species were collected in October (postmonsoon). Of these total collected mosquitoes, *An. annularis* was the highest with 140 in number (30.4%) followed by *An. maculatus* 81 (17.6%), *An. minimus* 46 (10%), *An. phillipinensis* 26 (5.7%), etc. During February (cool season), 138 *Anopheles* mosquitoes with 9 species were collected from Thayetpininn Village, where *An. maculatus* 49 (35.5%) was predominant and *An. minimus* 11 (8%) was also collected. There were 229 *Anopheles* mosquitoes included 6 species were collected in April (hot season). During May (premonsoon), the prevalence of primary vector *An. minimus* 71 (14.8%) was the most predominant and highest compared with other seasons. Secondary

Table 1. Seasonal prevalence of *Anopheles* mosquitoes caught in Thayetpininn Village, PyinOoLwin Township

<i>Anopheles</i> mosquitoes	October (postmonsoon) No. (%)	February (cool season) No. (%)	April (hot season) No. (%)	May (premonsoon) No. (%)	July (midmonsoon) No. (%)
<i>An. minimus</i>	46(10.0)	11(8.0)	26(11.3)	71(14.8)	59(11.6)
<i>An. maculatus</i>	81(17.6)	49(35.5)	94(41)	261(54.4)	216(42.6)
<i>An. annularis</i>	140(30.4)	13(9.4)	18(7.9)	25(5.2)	64(12.6)
<i>An. philippinensis</i>	26(5.7)	10(7.2)	5(2.2)	7(1.5)	21(4.1)
<i>An. varuna</i>	65(14.1)	26(18.8)	84(36.7)	65(13.5)	21(4.1)
<i>An. Splenditus</i>	0(0)	0(0)	0(0)	23(4.8)	39(7.7)
<i>An. willmori</i>	10(2.2)	0(0)	0(0)	28(5.8)	33(6.5)
<i>An. stephensi</i>	0(0)	8(5.8)	0(0)	0(0)	18(3.6)
<i>An. subpictus</i>	4(0.9)	0(0)	0(0)	0(0)	0(0)
<i>An. vagus</i>	3(0.6)	0(0)	0(0)	0(0)	9(1.8)
<i>An. rasayi</i>	63(13.7)	0(0)	0(0)	0(0)	0(0)
<i>An. jamesii</i>	10(2.2)	2(1.5)	0(0)	0(0)	0(0)
<i>An. tessellatus</i>	4(0.9)	0(0)	0(0)	0(0)	7(1.4)
<i>An. barbirostris</i> gp.	1(0.2)	7(5.1)	0(0)	0(0)	15(3.0)
<i>An. hyrcanus</i> gp.	7(1.5)	12(8.7)	2(0.9)	0(0)	5(1.0)
Total	460(100)	138(100)	229(100)	480(100)	507(100)

vector *An. maculatus* 261 (54.4%) was also found to be the most abundant compared with other seasons. In July (monsoon), 507 numbers of mosquitoes were collected and 12 *Anopheles* species were identified.

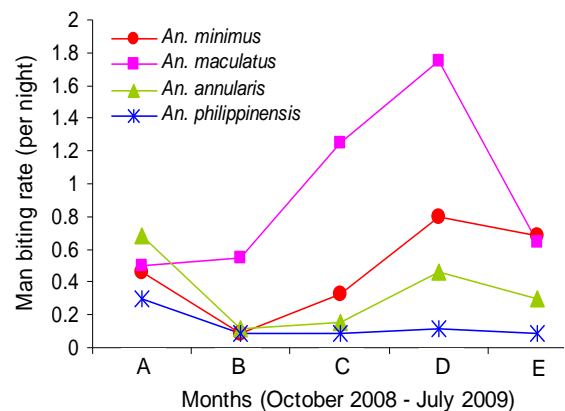
The behaviour of biting habits varies considerably between species with respect to time, place and host preference. In this study, most of the *Anopheles* vectors were fed mainly on cattle as well as on man and they preferred to be fed on man outdoor than indoor collection. Mosquitoes caught were presented in Table 2.

Table 2. Total number of *Anopheles* mosquitoes caught in Thayetpininn Village, PyinOoLwin Township (October 2008 - July 2009)

<i>Anopheles</i>	No. of mosquitoes (%)			
	Indoor	Outdoor	Betnet	
			Human bait	Cattle bait
<i>An. minimus</i>	14 (26.42)	39 (73.58)	19 (11.88)	141 (88.12)
<i>An. maculatus</i>	26 (17.69)	121 (82.31)	50 (9.03)	504 (90.97)
<i>An. annularis</i>	7 (13.46)	45 (86.54)	17 (8.17)	191 (91.83)
<i>An. philippinensis</i>	1 (4.55)	21 (95.45)	6 (12.77)	41 (87.23)

It was calculated that the peak density of *An. minimus* was 0.8 mosquitoes per man per night in May (premonsoon).

The highest man biting rate of *An. maculatus* was 1.75 in May and that of *An. annularis* was 0.68 in October. Man biting rate of *An. philippinensis* was lower compared to other *Anopheles* mosquitoes (Fig. 1).

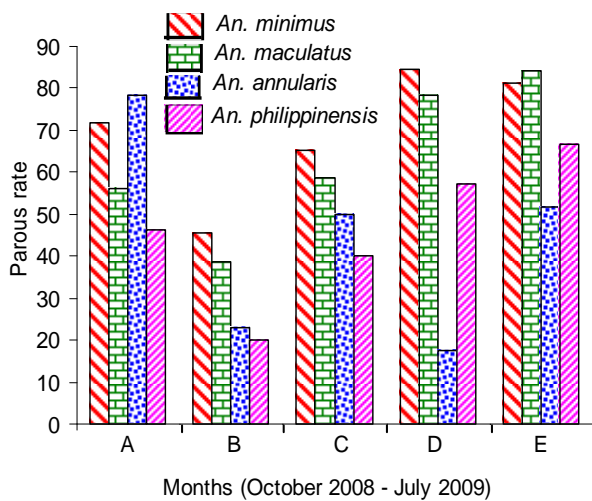


A = October (postmonsoon) B = February (cool season)  
C = April (hot season) D = May (premonsoon)  
E = July (monsoon)

Fig. 1. Seasonal man biting rates (MBR) of *Anopheles* in Thayetpininn Village, PyinOoLwin Township, 2008 - 2009

Salivary glands of a total of 163 (76.5%) *An. minimus* were dissected and examined for the presence of sporozoites. However, dissected *An. minimus* mosquitoes did not exhibit any infected sporozoite during the study period.

Figure 2 shows parous rate of four species (*An. minimus*, *An. maculatus*, *An. annularis*, *An. philippinensis*) during the study period.

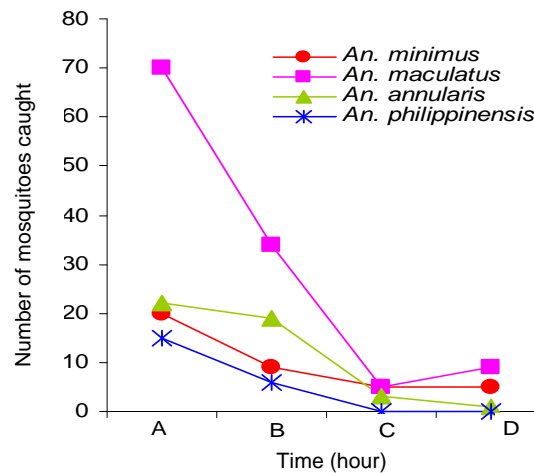


A = October (postmonsoon)    B = February (cool season)  
 C = April (hot season)        D = May (premonsoon)  
 E = July (monsoon)

Fig. 2. Parous rates of *Anophele* mosquitoes in Thayetpininn Village during the study period

For identification of mosquito breeding sites, larval surveys were conducted in all seasons. In this study, three breeding sites for *Anopheles* mosquitoes were categorized as slow running stream in small jungle, rice fields and water pocket. In larval surveys, *An. minimus* larvae were collected from slow running stream under shaded area in October, April, May and July. During October (postmonsoon) *An. minimus* larvae were also found in rice fields. However, *An. minimus* larvae were not found in water pocket. Then, *An. maculatus* and *An. varuna* immatures were found from slow running stream in all seasons.

Furthermore, *An. maculatus* larvae were found in water pocket during October and July. Then, *Anopheles annularis* and *An. Philippinensis* larvae were collected from slow running stream and rice fields during October. During October, the breeding site of *An. barbirostris* gp. larvae was found in slow running stream.



A = 18:00 - 21:00 (first quarter)  
 B = 21:00 - 24:00 (second quarter)  
 C = 24:00 - 03:00 (third quarter)  
 D = 03:00 - 06:00 (fourth quarter)

Fig. 3. Biting patterns of *Anopheles* mosquitoes on human bait outdoor during the study period (October 2008 - July 2009)

The biting patterns of *Anopheles* mosquitoes fed on human bait outdoor during the study period are shown in Fig. 3. The peak biting activities of *An. minimus*, *An. maculatus*, *An. annularis* and *An. philippinensis* were found in first quarter of night during the study period.

## DISCUSSION

The entomological indicator of a mosquito vector depends on many factors. Among them, more important factors are the total number of mosquitoes, the degree of their contact with man, their life span and their susceptibility to infection with Plasmodia.<sup>6</sup>

The prevalence of total 1814 *Anopheles* vectors with 15 species caught during the study period was determined. Among 15 species, one of the important primary vectors was *An. minimus* and others were secondary and non-vectors. *An. minimus* was prevalent in the study area throughout the year. Thus, *An. minimus* has a wider distribution and higher densities as well as a longer seasonal prevalence.<sup>7</sup>

In the present study *An. minimus*, *An. maculatus*, *An. annularis* and *An. philippinensis* were found to bite more cattle than human. In Thailand, *An. minimus* was also found to be zoophilic<sup>3, 8</sup> and the animal biting densities were high throughout the year.<sup>3</sup> Most of the *Anopheles* vectors such as *An. minimus*, *An. maculatus*, *An. annularis* and *An. philippinensis* preferred to feed on human outdoor than on human indoor. *An. minimus* was predominantly exophilic, the outdoor biting rate being about 3 times of the indoor biting rate.<sup>9</sup>

In the present study, *An. minimus* larvae were collected mainly from slow running stream in small jungle except in February and *An. minimus* larvae were collected in rice fields during October only. *An. maculatus* larvae were collected from slow running stream throughout the study period. Another study conducted in Wetwun, Maymyo Township showed that *An. minimus* larvae were found in small running streams (sometimes located on the roadside) at points where water flow was extremely sluggish and edges of stream covered with vegetation.<sup>7</sup> *An. philippinensis* larvae were collected in rice fields during October. *An. philippinensis* was found to breed in ponds, cultivated or derelict paddy fields, tanks and swamps.<sup>10</sup> In our study, all of rice fields were dried up before harvest and water pocket also dried up in cool and hot seasons.

In the present study, parous rate of *An. minimus* was the highest in premonsoon. Man biting rate of *An. minimus* in premonsoon was higher than that of other periods. Man biting rate and parous rate of *An. maculatus* were high in premonsoon. In upper Myanmar, April and early May are arranged as dry hot season. However, as vector densities are based on seasonal climate condition, their seasonal activities are varied. Due to the possibility of some rain fall in May, mosquito densities were higher in this month. Parity determination in research and control programme is

associated with determining the condition of vectors ovary. It is important to know the parity rate of the vector responsible for disease transmission and the timing in which parity peaks.

In the present study, the sporozoite rate could not be calculated. Dissected *Anopheles* mosquitoes did not exhibit any infected sporozoite during the study period. It might be due to two facts; one fact was lower density of collected *Anopheles* mosquitoes in the present study and the other was less chance to see sporozoite in salivary gland of infected *Anopheles* mosquitoes according to other findings. During the longitudinal study conducted in Phado, the sporozoite rate of *An. minimus* was found to be 0.43% (3/699).<sup>7, 9</sup> The sporozoite rate of *An. minimus* based on ELISA results was 2.98% (2/67) in Thabyewa Village, Oktwin Township.<sup>3</sup>

The results of the biting cycle on human outdoor showed that *An. minimus* were early night biters especially in the first quarter of night. The biting cycle on human outdoor showed that the secondary vectors such as *An. maculatus*, *An. annularis* and *An. philippinensis* were also early night biters especially in the first quarter of night. In upper central Thailand, however, *An. minimus* tended to be an early evening biter of humans in the cool dry season and an early morning biter of humans in the wet season, thereby increasing the chance of human vector contact.<sup>8</sup>

### Conclusion

*An. minimus* (primary vector) often fed outdoors or have feeding peaks before most people go to bed. The peak biting time for most *Anopheles* mosquitoes in this study ranged in first quarters of night (06:00-09:00) hour. Thus, an effective means of personal protection, which can be used outdoors and/or before people go to bed, will be of significant value. The results of this study are expected to be useful in personal protection against mosquitoes in this area.

## ACKNOWLEDGEMENT

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**Analysis of Haemoglobin Variants and HbA1c Level in  
Type 2 Diabetes Mellitus Patients in Yangon**

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In Myanmar, overall prevalence rate of diabetes mellitus is 8-12% in general population. Haemoglobin variants are HbS, HbC, HbE, HbH and Hb Barts. Among them, haemoglobin E is one of the commonest abnormal haemoglobin variants in the world. Haemoglobin A1c (HbA1c) is a marker of long-term glycaemic control in patients with diabetes mellitus and is directly related to diabetic complications. A cross-sectional, descriptive study was performed to analyze the haemoglobin variants and HbA1c level in type 2 diabetes mellitus patients in Yangon Division. A total of 381 type 2 diabetes mellitus patients including 83 males and 298 females were tested for haemoglobin types by isoelectric focusing gel electrophoresis (IEF) method and HbA1c% by using high performance liquid chromatography (HPLC). HbA (normal Hb) was detected in 326 diabetic patients (85.6%), HbEA in 51 patients (13.4%) and HbEE was detected in 4 patients (1.04%). The mean HbA1c% was 6.96% in male and 7.24% in female diabetic patients. The mean HbA1c value was 7.44% in HbA type, 3.65% in HbEE type and 5.78% in HbEA type, respectively. The mean HbA1c value was significantly lower in males having HbEE compared to female subjects with this type i.e 2.5% vs. 4.03% ( $p < 0.001$ ). Mean HbA1c value was significantly decreased in abnormal haemoglobin variants like HbEE and EA types in the diabetic patients. Diabetic patients with lower HbA1c value (<4%) should be investigated for detection of haemoglobin variants (abnormal Hb type) by IEF method. Assessment of HbA1c level is an effective management for glycaemic control of type 2 diabetic patients.

## INTRODUCTION

In the world, prevalence of diabetes was estimated to be 171 million among people of more than 20 years of age in 2000 and it will be increasing up to 334 million of people globally in 2030. The prevalence of diabetes for all age groups was estimated to be 2.8% in 2000 and 4.4% in 2030 in the world. The most common age group was >65 years in both sexes globally. In developing countries, the majority of diabetic patients are in the age group of 45-64 years and the common age group of

diabetes is >64 years in the developed countries.<sup>1</sup> Diabetes mellitus is a medical condition in which the body does not produce or properly use insulin, a polypeptide hormone produced from beta cell of the islets of Langerhans of the pancreas. It regulates the metabolism of glucose and other nutrients. Type 2 diabetes occurs mainly in adults over 40 years of age with or without being overweight. It is caused by insulin deficiency, genetically inherited or due to resistance by the tissues of the body.<sup>2</sup> In Myanmar, diabetes is one of the commonest non-communicable diseases

and overall prevalence rate was 8-12%. The prevalence rate for gender was 11.5% in male and 12.6% in female according to data of Health in Myanmar 2011, Ministry of Health.<sup>3</sup>

Haemoglobin variants are HbS, HbC, HbE, HbH and Hb Barts. Among them, haemoglobin E is one of the commonest abnormal haemoglobin variants in South East Asia. In Myanmar, heterozygous HbE (HbEA) prevalence is 11% to 24% of general population and it associates with influence of ethnic groups.<sup>4</sup> HbE is caused by substitution of glutamic acid for lysine at position 26 of beta globin gene.<sup>5</sup>

Haemoglobin A1c (HbA1c) is a marker of long-term glycemic control in patients with diabetes mellitus and is directly related to diabetic complications. HbA1c is a form of irreversible non-enzymatic glycation at one or both NH<sub>2</sub> terminal valines of the  $\beta$  chains.<sup>6</sup> HbA1c represents both fasting and postprandial glycemic control states in diabetic patients. It is used for diagnosis of diabetic control because it has 65% sensitivity and 94% specificity.<sup>7</sup> This study was performed to identify the types of haemoglobin (Hb variants) and determine the HbA1c value in type 2 diabetes mellitus patients in Yangon.

#### *General objective*

- To analyze the haemoglobin variants and haemoglobin A1c value in type 2 diabetes mellitus patients in Yangon.

#### *Specific objectives*

- To find out the types of haemoglobin (haemoglobin variants) in type 2 diabetes mellitus patients taking diabetic treatment in Yangon
- To determine haemoglobin A1c value in these type 2 diabetic patients
- To find out the association between the types of haemoglobin variants and HbA1c value in type 2 diabetes mellitus patients

## **MATERIALS AND METHODS**

A cross-sectional, descriptive study was conducted in known type 2 diabetes mellitus patients who were taking diabetic treatment at Yangon General Hospital (YGH) and North Okkalapa General Hospital (NOGH).

A total of three hundred and eighty-one known case of type 2 diabetes mellitus patients were recruited and explained about the benefits from outcomes of this research study and how it could help in the treatment. After being approved by the Institutional Ethical Review Committee, Department of Medical Research (Lower Myanmar), informed written consents were obtained from these patients.

Three milliliters of whole blood from an ante-cubital vein of each subject were collected into ethylene diamine tetraacetate (EDTA) containing K3 test tube and mixed thoroughly and kept with ice pack for 24 hours until testing in the Pathology Research Division.

All samples were tested for haemoglobin variants by using isoelectric focusing electrophoresis (IEF) and HbA1c value was determined by using ion exchanged high performance liquid chromatography (HPLC) analyzer. Whole blood (10  $\mu$ l) was mixed with 10 $\mu$ l of 1% saponin solution for at least 3 hours at room temperature before running the isoelectric focusing electrophoresis at pH 6.0-6.2 to identify the different types of hemoglobin in the blood by separation of globin chains.

Whole blood (20  $\mu$ l) was mixed with 1000  $\mu$ l of distilled water for at least 3 hours at room temperature before determining HbA1c by HPLC analyzer. The results obtained throughout the study were recorded according to the proforma and all data were calculated for odds ratios (Ors), p-value and confidence interval (CI) by using the equality of populations (Kruskal-Wallis rank sum test) in this study.

## RESULTS

### Characteristics of subjects

In this study, total number of type 2 diabetic patients was 381 including 83 male and 298 female. Type 2 diabetes in this study was 3.5 times higher in female than in male subjects (21.8% in male and 78.2% in female). The mean age of type 2 diabetic patients was  $55.15 \pm 9.99$  years in both sexes. The commonest age group was found to be 60-85 years (50.60%) in male and 40-59 years (61.74%) in female diabetic patients. The least common age group was 20-39 years (5.77%, 22 out of 381 cases) in both genders (Table 1).

Table 1. Distribution of type 2 diabetic patients by age groups and gender

Age (years)	Male No. (%)	Female No. (%)	Total cases No. (%)
20 - 39	5(6.02)	17(5.70)	22(5.77)
40 - 59	36(43.37)	184(61.74)	220(57.74)
60 - 85	42(50.60)	97(32.55)	139(36.48)
Total	83(21.78)	298(78.21)	381(100)

### Types of haemoglobin variants

Hemoglobin 'A' (normal hemoglobin) was detected in 326 cases (85.6%) of the type 2 diabetic patients comprising 71 male and 255 female subjects. HbEA was found in 51 out of 381 cases (13.4%) (11 males and 40 females) and HbEE was identified in 4 out of 381 subjects (1.04%) (1 male and 3 females) by using the isoelectric focusing gel electrophoresis test. In this study, Hb EA variant was higher in females than in males (78.4% vs. 21.6%) and it was the same with Hb EE variant (75% vs. 25%).

Table 2. Distribution of different haemoglobin types by sex in type 2 diabetic patients

Haemoglobin types	Male (%)	Female (%)	Total (%)
Haemoglobin A	71(21.8)	255(78.2)	326(85.56)
Haemoglobin EA	11(21.6)	40(78.4)	51(13.38)
Haemoglobin EE	1(25.0)	3(75.0)	4(1.04)
Haemoglobin variants (HbS, HbC, HbH & Hb Barts)	-	-	-
Total	83(21.78)	298(78.21)	381(100)

Other haemoglobin variants like HbS, HbC, HbH and Hb Barts were not detected in any

of the type 2 diabetic patients by using the isoelectric focusing gel electrophoresis (Table 2).

The Hb EA variant was highest in 40-59 years age group (14.5%) and HbEE variant was commonest in 60-85 years age group (2.2%) (Table 3).

Table 3. Distribution of haemoglobin types by age groups in type 2 diabetic patients

Age(years)	HbA No. (%)	HbEA No. (%)	HbEE No. (%)	Total cases No. (%)
20 - 39	21(95.5)	1(4.5)	0(0.0)	22(5.77)
40 - 59	187(85.0)	32(14.5)	1(0.5)	220(57.74)
60 - 85	118(84.9)	18(12.9)	3(2.2)	139(36.48)
Total	326(85.56)	51(13.38)	4(1.04)	381(100)

### Determination of haemoglobin A1c (HbA1c) value in type 2 diabetes mellitus

The mean value of HbA1c was 7.18% in all age groups and the normal reference of HbA1c was 4-7% in non-diabetic patients. The minimum HbA1c value was 2.5% and maximum value was 15.6% in this study. It was not significantly different in the different age groups and sexes of type 2 diabetic patients by using Kruskal-Wallis rank sum test ( $p=0.4337$  and  $0.3835$ ) (Table 4, 5).

Table 4. Distribution of haemoglobin A1c (HbA1c) values in different age groups of type 2 diabetes cases

Different Age groups (years)	Value of HbA1c				
	Mean (%)	Median (%)	Standard deviation	Minimum (%)	Maximum (%)
20 - 39	7.43	6.95	2.7	4.2	13.1
40 - 59	7.34	6.8	2.3	2.6	15.6
60 - 85	6.86	6.7	2.1	2.5	13.9
Total	7.18	6.7	2.3	2.5	15.6

Normal reference value of Hb A1c = 4-7% (HPLC test)  
 $p= 0.4337$  (Kruskal-Wallis rank sum test)

Table 5. Distribution of haemoglobin A1c (HbA1c) values by sex in type 2 diabetes mellitus patients

Different Sex	Value of HbA1c				
	Mean (%)	Median (%)	Standard deviation	Minimum (%)	Maximum (%)
Male	6.96	6.4	2.6	2.5	15.6
Female	7.24	6.8	2.1	2.6	15.5

$P= 0.3835$  (Kruskal-Wallis rank sum test)



The mean value of HbA1c was 7.44% in haemoglobin type A, 5.78% in haemoglobin type EA and 3.65% in haemoglobin type EE, respectively, in this study. Mean HbA1c value was highest in haemoglobin A type (7.44%) and lowest in haemoglobin EE variant (3.65%). The mean HbA1c value was significantly different between the haemoglobin variants in type 2 diabetic patients according to Mann-Whitney test and student 't' test  $p < 0.001$  (Table 6).

Table 6. Results of HbA1c value and different types of haemoglobin (Hb variants) in type 2 diabetes mellitus patients

Different haemoglobin types	Value of HbA1c				
	Mean (%)	Median (%)	Standard deviation	Minimum (%)	Maximum (%)
HbA	7.44	6.95	2.2	3.4	15.6
HbEA	5.78	5.7	1.8	2.6	9.9
HbEE	3.65	3.3	1.4	2.5	5.5
Total	7.18	6.7	2.3	2.5	15.6

Table 7. Results of mean HbA1c value and mean fasting blood sugar level in different haemoglobin types of type 2 diabetes mellitus patients

Different haemoglobin types	Mean HbA1c value (%)	Mean fasting blood sugar value (mg%)
HbAA	7.44	168
HbEA	5.78	162
HbEE	3.65	260

In this study, the highest mean fasting blood sugar FBS (260 mg%) was found in HbEE type and the lowest FBS was observed (162mg%) in HbEA type. The mean FBS in normal HbAA type was 168 mg%. The mean HbA1c value was lowest in HbEE type (3.65%) and the highest in HbAA type (7.44%) and 5.78% in HbEA type determined by HPLC (Table 7).

## DISCUSSION

In this study, the commonest age group of diabetes mellitus was 40-59 years (57.74%) in female and 60-85years (50.56%) in male. In the world, the commonest age group is more than 65 years in both genders.<sup>1</sup> The prevalence of type 2 diabetic mellitus was higher in females than in males in all age

groups in this study (78.2% vs. 21.8%). In the world, prevalence rate of diabetes was slightly higher in male than female at under 60 years age and female was higher than male in over 60 years age.<sup>1</sup> Other studies revealed that type 2 diabetic mellitus showed sex variation depending on age. The commonest HbAA type (normal Hb) was detected in 85.6% of type 2 diabetic patients. HbEA and HbEE (Hb variants) were also noted in 13.4% and 1.04% of patients in this study. In previous research study of Myanmar, HbE trait was detected in 20% of blood donors from National Blood Center in Myanmar.<sup>8</sup> HbE carrier prevalence rate was 19.09% (18.09% in HbEA and 1% in HbEE) in healthy school children (5-12 years) in Yangon.<sup>9</sup> Other haemoglobin variants such as HbS, HbC, HbF, HbH and Hb Barts were not detected by IEF in this study. HbE is one of the commonest hemogloninopathies in the world that is a heterogeneous group of disorders whose phenotypes range from asymptomatic to severe. The prevalences of HbEA (HbE trait) and HbEE in the diabetic clinic were very high i.e 38.2% and 9% in Surin Hospital, Thailand.<sup>10</sup>

The mean value of HbA1c was 7.4% in HbAA type, 5.78% in HbEA type and 3.65% in HbEE type, respectively, in type 2 diabetic patients. The mean HbA1c value was 6.96% in male and 7.24% in female subjects in this study. The mean fasting blood sugar level (FBS) was 168 mg% in HbAA and 162 mg% in HbEA type but it was very high FBS level (260 mg%) in HbEE type of diabetic patients. The mean HbA1c value was 7.51% and mean fasting blood sugar was 148 mg% in type 2 diabetic patients at one research study in Sri Lanka.<sup>11</sup> The mean HbA1c value was significantly different ( $p < 0.001$ ) in haemoglobin variants including HbEA and HbEE in this study. IEF is a electrophoretic technique with excellent resolution that has been used to identify the Hb variants such as HbC, HbE, HbA, HbF, HbS, HbD, HbG according to resolution and migration of hemoglobin in a

pH gradients. HbA1c determination is one of the useful laboratory test for glycemic control in diabetic patients. HbA1c determination could be interfered by Hb variants with HPLC method. The best methods for determining HbA1c in diabetic patients with haemoglobin variants are immunoassays and boronate affinity chromatography.<sup>12</sup> HbA1c concentration shows poorer correlation with blood glucose in diabetic patients having haemoglobin variants.<sup>13</sup> Diabetic mellitus patients with lower limit of reference HbA1c value (<4%) should be investigated for detection of haemoglobin variants by isoelectric focusing electrophoresis or cellulose acetate electrophoresis. Assessment of HbA1c level is an effective management for glycemic control in type 2 diabetic patients.

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***In-vitro* Antimicrobial Resistance among Predominant Bacterial Pathogens Isolated from Septic Abortion Cases in North Okkalapa General Hospital**

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Septic abortion is a major health challenge leading to maternal morbidity and mortality in developing countries. Antibiotic therapy contributes to an important role along with the removal of septic focus by evacuation of retained products of conception. A cross-sectional descriptive study was carried out to determine the bacteriological spectrum and antibiotic susceptibility pattern of septic abortion cases admitted to North Okkalapa General Hospital during 2009-2010. Blood and endocervical swab cultures were performed to identify bacterial pathogens using standard microbiological techniques. The isolates were subjected to antibiotic susceptibility tests using the disc diffusion method. Of 160 septic abortion cases, pathogenic aerobic bacteria were isolated in 54.4% (87/160) and septicaemia proven by blood culture was found in 17.5% (28/160). *Staphylococcus aureus* was the most prevailing bacterial pathogen comprising 36.8% (32/87) of culture-positive cases. *Escherichia coli* was the second most commonly isolated bacterial pathogen accounting for 29.9% (26/87). Antibiotic susceptibility of *Staphylococcus aureus* indicated 72-94% sensitivity to amoxicillin-fluocloxacillin, cefoperazone-sulbactam, cefipime and piperacillin-tazobactam. However, 63-97% resistance to ceftriazone, amoxicillin-clavulanic acid and penicillin was found. Methicillin-resistant *Staphylococcus aureus* strains were found in 20% (5/25) of *Staphylococcus aureus* isolates. *Escherichia coli* isolates showed 75-88% sensitivity to cefoperazone-sulbactam, cefotaxime, ceftazidime, piperacillin-tazobactam and cefipime; and 58-96% resistance to ceftriazone, ofloxacin, ciprofloxacin and penicillin. Extended-spectrum beta-lactamase producing *Escherichia coli* were found in 11.5% (3/26) of *Escherichia coli* isolates. The present study highlighted the emergence of multidrug-resistant bacterial pathogens and provided the information for development of effective updated antibiotic regime for septic abortion.

## INTRODUCTION

Septic abortion is a life threatening medical problem all over the world and it accounts for significant proportion of all the gynaecological emergency admissions. It was reported that maternal mortality from abortion is high in 18 specialist hospitals in Myanmar and more than 50% of maternal mortality was due to septic abortion.<sup>1</sup> Haemorrhage and sepsis are major contri-

buted causes for morbidity and mortality. Management of severe sepsis includes eradication of infection, improvement of general condition and surgical intervention to remove the septic foci. Eradication of infection depends upon antibiotic selection based on culture and sensitivity. The correct choice of antibiotics is of paramount importance and it greatly improves the survival and reduces the complications of both immediate and long-term consequences.

The organisms most frequently encountered in the genital tract were coliform bacilli, Enterococci, *Bacteriodes* species, Clostridium, Peptostreptococcus and other organisms indigenous to the genital tract. Among them, microorganisms most frequently found in septic abortion were *Bacteriodes* species, anaerobic Streptococci, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Clostridium perfringens*, *Escherichia coli*, *Proteus* species, *Gardnerella vaginalis* and *Listeria monocytogenes*.<sup>2</sup>

Knowledge of bacterial pathogens and antibiotic sensitivity among septic abortion cases is urgently required to improve patient care by selecting the most suitable antibiotics. A clinical profile of septic abortion was studied in North Okkalapa General Hospital (NOGH) in 1995 and indicated that *E. coli* was the most commonest offender.<sup>3</sup>

A study in 1999 revealed that the more frequently found infecting organisms were *Klebsiella* and *Pseudomonas aeruginosa*. Overall organisms were found to be highly sensitive to cephalosprins (cefotaxime or ceftriaxone) and less sensitive to quinolones (pefloxacin or ciprofloxacin).<sup>4</sup>

It was reported that gram-negative coliform *E. coli* was the commonest offender and gram-positive coccus *Staphylococcus aureus* was the second among septic abortion cases in NOGH.<sup>5</sup> Antibiotic sensitivity pattern showed ceftriazone, ciprofloxacin, amikacin and gentamicin were the most effective antibiotics against gram-negative bacilli and clindamycin, oxacillin, ciprofloxacin and gentamicin were effective against gram-positive cocci.

The burden of antimicrobial resistance is increasing among various antimicrobial agents and in different settings over the world. Multidrug-resistant hospital pathogens are emerging and can lead to increased morbidity and mortality. With clinical indications of possible change in the predominant causal microorganisms and emergence of resistant strains to current antibiotic regime, the present study was

carried out to determine the bacteriological spectrum and antibiotic sensitivity pattern of septic abortion cases in NOGH in order to improve the treatment outcomes and reduce complications.

## MATERIALS AND METHODS

A cross-sectional descriptive study was carried out on 160 septic abortion cases during 2009 and 2010 at NOGH.

### *Selection of patients*

Abortion is variously defined as the expulsion or extraction of a fetus (embryo) weighing less than 500 g equivalent to approximately 20-22 weeks gestation,<sup>6</sup> or as termination before 24 weeks of gestation with no evidence of life.<sup>7</sup> Septic abortion is an abortion associated with infection and complicated by fever, endometritis and parametritis.

### *Operation definition for septic abortion*

Patients with amenorrhoea of less than 24 week gestation with -

- a temperature of 100°F (37.8°C) and above
- suprapubic pain, and
- vaginal bleeding with foul smelling discharge

### *Procedure*

Relevant history taking was done according to the proforma. Specimens were taken and sent to the Bacteriology Research Division, Department of Medical Research (LM) for culture and antibiotic sensitivity.

### *Specimen collection*

Two endocervical swabs were taken for detection of aerobic pathogenic bacteria. Each swab was inoculated into Amies transport medium and Stuart's transport medium for aerobic culture.

Five milliliters of blood were collected under aseptic condition. The blood was collected before the administration of parental antibiotics. Five milliliters of blood

were inoculated into blood culture bottle containing tryptic soy broth with sodium polyanethol sulfonate for aerobic culture.

The pathogenic bacteria were identified according to the method described in the WHO Manual and Cowen and Steel's Manual for Identification of Medical Bacteria.<sup>8, 9</sup>

#### *Detection of Neisseria gonorrhoeae*

The endocervical swab in Amies transport media was inoculated onto Modified Thayer Martin agar and chocolate agar and inoculated at 35°C up to three days in the carbon dioxide incubator. Positive cultures from chocolate and Thayer Martin agar were identified by colony morphology, Gram staining, positive oxidase reaction, and the phabedact agglutination.

#### *Aerobic culture procedure*

An endocervical swab in Stuart's transport media was inoculated onto mannitol salt agar, blood agar, McConkey's agar and nutrient agar and incubated at 37°C overnight. Positive cultures were identified by colony morphology, Gram staining and biochemical reaction.

#### *Blood culture procedure*

Tryptic soy broth with blood was incubated at 37°C up to 7 days. Blood culture bottles were examined at 14-17 hours and then everyday for up to 7 days. Blood cultures showing turbidity or lysis of erythrocytes were subcultured to identify bacterial pathogens.

#### *Antibiotic susceptibility testing*

The confirmed bacterial isolates were tested for antibiotic susceptibility by disc diffusion test by Modified Kirby Bauer Method as recommended by Clinical Laboratory Standard Institute (CLSI).

#### *Detection of extended-spectrum beta-lactamase (ESBL) producers*

Ceftazidime and cefotaxime were included in the primary panel for screening potential

ESBL producers. Potential ESBL producers were undergone confirmatory testing by a double disk synergy test (DDST). Ceftazidime, cefotaxime and ceftriaxone 30 µg each were placed at a distance of 15mm edge to edge from a centrally placed amoxicillin-clavulanic acid disk containing 20 µg of amoxicillin + 10 µg of clavulanic acid. ESBL production is inferred if the inhibition zone around the test antibiotic disk increases towards the amoxicillin-clavulanic acid disk.

#### *Detection of methicillin-resistant Staphylococcus aureus (MRSA)*

MRSA was detected by using oxacillin (a semi-synthetic penicillin similar to methicillin) disc. MRSA showed survival in the presence of oxacillin.

#### *Ethical consideration*

This study was approved by the Institutional Ethical Review Committee of Medical Research Involving Human Subjects, Department of Medical Research (LM).

#### *Data analysis*

The data were analyzed using SPSS version 17.0. Descriptive statistics were used to determine demographic characteristics. Means and ranges were used to describe the demographic data and number and percentage were calculated for categorical data.

## **RESULTS**

#### *Demographic characteristics of study population*

In the present study, septic abortion patients were ranged from 18-43 years of age. Unmarried women accounted for 1.9% of the study population. The average gestation age of septic abortion cases was 11.8 weeks and most of the cases were multiparity women. About 18% showed previous history of abortion (Table 1).

Table 1. Demographic characteristics of septic abortion cases (n=160)

Characteristics	Frequency	%	Mean±SD	Range
<b>Age in completed years</b>				
≤ 24	46	28.8	29±6.6	18-43
25-39	106	66.2		
≥ 40	8	5.0		
<b>Marital status</b>				
Unmarried	7	1.9	11.8±6.4	1-24
Married	146	91.2		
Separated/divorced & widowed	7	1.9		
<b>Gestation age (weeks)</b>				
1-6	27	17.9	2.6±1.9	1-9
7-12	81	50.6		
13-18	23	14.4		
19-24	23	14.4		
Not known	6	3.7		
<b>Obstetric history</b>				
<b>Gravida</b>				
G1 to G3	115	71.2	1.4±1.5	0-8
G4 to G6	39	24.4		
G7 to G9	6	3.8		
<b>Parity</b>				
P0 to P2	131	81.8	2.6±1.9	1-9
P3 to P5	27	16.9		
P7 to P8	2	1.3		
No. of previous abortion	29	18.1	1.4±1.5	0-8
Use of contraception	84	52.5		

*Pathogenic aerobic bacteria isolated from septic abortion cases*

Of 160 septic abortion cases, pathogenic aerobic bacteria were isolated in 54.4% (87/160) comprising 87 isolates from endocervical swabs and 28 isolates from blood culture. Thus, blood culture-proven septicemia accounted for 17.5% (28/160) of septic abortion cases (Fig. 1).

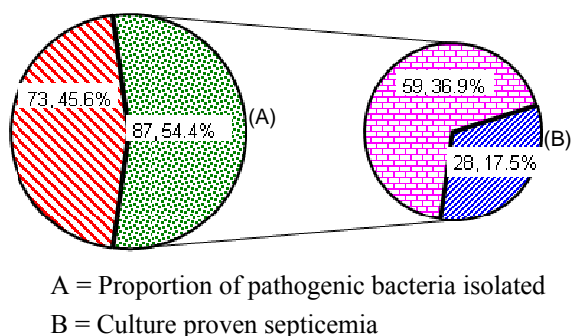


Fig.1. Proportion of isolated pathogenic aerobic bacteria among 160 septic abortion cases

All blood culture-positive cases were endocervical swab culture positive and isolated bacteria were the same. *S. aureus* was the most prevailing bacterial pathogen comprising 36.8% (32/87) of culture-positive cases. *E. coli* was the second most

commonly isolated bacterial pathogen accounting for 29.9% (26/87) (Table 2). Mixed infections were found in 4.4% (7/160) (Table 3).

Table 2. Bacterial pathogens isolated from septic abortion cases

Isolated bacteria	Frequency			%
	Single infection	Mixed infection	of culture-positive cases	
<i>Staphylococcus aureus</i>	30	2	32	36.8
<i>Escherichia coli</i>	24	2	26	29.9
<i>Proteus mirabilis</i>	12	-	12	13.8
<i>Citrobacter freundii</i>	6	2	8	9.2
<i>Klebsiella spp.</i>	3	1	4	4.6
<i>Neisseria gonorrhoeae</i>	1	2	3	3.4
<i>Coagulase-negative staphylococci</i>	1	1	2	2.3
Total	77	10	87	100

Table 3. Types of bacterial pathogens isolated in mixed infection

Bacteria isolated in mixed infections	No. of cases
<i>Staphylococcus aureus</i> + <i>Citrobacter freundii</i>	2
<i>Staphylococcus aureus</i> + <i>Escherichia coli</i>	2
<i>Escherichia coli</i> + <i>Neisseria gonorrhoeae</i>	1
<i>Citrobacter freundii</i> + <i>Neisseria gonorrhoeae</i>	1
<i>Klebsiella spp</i> + <i>Coagulase-negative staphylococci</i>	1
Total	7

*Antimicrobial susceptibility pattern of aerobic bacterial pathogens isolated from septic abortion cases*

The most commonly identified *S. aureus* isolates were found to be 93.7% (30/32) sensitive to piperacilin-tazobactam. Highly sensitive antimicrobial drugs were cefipime (28/32, 87.5%), cefoperazone-sulbactam (26/32, 81.3%) and amoxicillin-fluocloxacillin (23/31, 74.1%). They were moderately sensitive to ceftazidime (20/32, 62.5%), ofloxacin (19/32, 59.3%), gentamicin (17/32, 53.1%), and ciprofloxacin and amoxicillin-clavulanic acid (16/32, 50% each). Highly resistant antibiotics were ceftriaxone (20/32, 62.5%), amikacin (25/32, 78.1%), and penicillin (31/32, 96.8%).

Five isolates that were found to be resistant to oxacillin (alternative of methicillin) were also resistant to many other antibiotics as well. Therefore, these *Staphylococcus aureus*

isolates could be regarded as methicillin-resistant *Staphylococcus aureus* (MRSA). MRSA isolates were also resistant to ceftriaxone, ceftazidime and gentamicin and piperacillin-tazobactam.

For *Escherichia coli* isolates, cefipime was the most sensitive drug (23/26, 88.4%) followed by piperacillin-tazobactam (22/26, 84.6%), ceftazidime (21/26, 80.8%), cefotaxime and cefoperazone-sulbactam (20/26, 75.8% each). It was moderately sensitive to amikacin, gentamicin and amoxicillin-fluocloxacillin. It was highly resistant to ceftriaxone (15/26, 57.6%), ofloxacin (20/26, 76.9%), ciprofloxacin (22/26, 84.6%) and penicillin (24/25, 96%). Extended-spectrum beta-lactamase producing *E. coli* were found in 11.5% (3/26) of *E. coli* isolates.

The *Citrobacter freundii*, *Proteus* species and *Klebsiella* species isolates were highly sensitive to amikacin, cefepime, cefoperazone-sulbactam, ceftazidime and piperacillin-tazobactam, 50% sensitive to ofloxacin and amoxicillin-fluocloxacillin, and totally resistant to ceftriaxone, ciprofloxacin, gentamicin and amoxicillin-clavulanic acid in this study. Two isolates of coagulase-negative *Staphylococci* were totally sensitive to amikacin, cefepime, cefoperazone-sulbactam, ceftazidime, gentamicin, ofloxacin, oxacillin and piperacillin-tazobactam. They were resistant to amoxicillin-clavulanic acid, amoxicillin-fluocloxacillin, ciprofloxacin and penicillin.

## DISCUSSION

In the present study, the majority of patients (66.2%) were in the age group of 25-39 years. In one study about two third of the unsafe abortions were performed in women aged between 15-30 years and 14% of all unsafe abortions were performed under 20 years.<sup>10</sup> Knowledge of age pattern is essential to a better understanding of the local situation and to tailoring interventions to prevent unsafe abortion.

It was reported that patients with abortion were young age ( $26\pm 6$  years), with mean parity of  $2\pm 2$  and 26% of them had their first pregnancy and 78% had their first abortion.<sup>11</sup> Abortion was more frequent between 9 and 12 weeks of pregnancy. In the present study, first trimester abortions were more common than second trimester abortions. It contributed to 17.9% in 1-6 weeks and 50.6% in 7-12 weeks of gestation. This finding is in line with the other studies.

The present study showed 86.6% were parity 0 to 2 group. In one study, abortion mostly occurred in parity 0-2 and other studies in Myanmar also reported similarly.<sup>12, 3, 13, 15</sup> Hence, the parity distribution of septic abortion occurred in parity 0-2 and it might be due to the fear of having pregnancy especially in young primigravid group who perceive abortion as illegal and unethical.

According to the present study, only 52.5% of patients used contraception. Contraceptive failure is an obvious reason for an unwanted pregnancy and most of them ended with unwanted abortions.

Of 160 septic abortion cases, only 54.4% were culture-positive cases. Only aerobic culture can be carried out in the present study, thus anaerobic infection cannot be ruled out as causal agents for septic abortion cases. Culture-proven septicemia cases were found in 17.5% of tested cases. Septicemia is one of the major complications and it is fatal. Patients with more severe manifestation of sepsis had higher incidence of positive blood culture. The principle pathogen responsible for septic shock in septic abortion was aerobic gram-negative coliform organism that produces endotoxin.<sup>14</sup>

Regarding the bacteriological profile, *Staphylococcus aureus* was the most common bacterial pathogen followed by *Escherichia coli*. Previous studies in NOGH, in 1999 and 2008 showed that

*E. coli* was the commonest followed by *S. aureus*.<sup>4,5</sup>

Staphylococci are the chief offenders of acute postabortal or puerperial pelvic sepsis. Septic shock may accompany overwhelming gram-positive bacterial infection because they produce an exotoxin. Coagulase-negative staphylococci (CoNS) are substantially less virulent than *S. aureus* and are opportunistic pathogens. They are nosocomial pathogens and related to medical procedures and practices. In septic abortion, *Staphylococcus epidermidis* is the common aerobic infecting organism.<sup>2</sup>

In endocervical swabs, mixed infections were also isolated. Bacteria make up a large proportion of the normal vagina flora. Therefore, it is not surprising that these organisms are frequently involved in septic abortion as indicated by the present findings. Mixed infections were isolated from endocervical samples only. This may be due to the fact that only one microorganism possibly invaded the blood stream and others do not.

In the present study, the culture positivity rate was higher in endocervical swabs than in blood cultures. This low isolation rate of blood culture may be due to the localized primary infection in pelvis or genital tract and there was no invasion of blood stream by bacteria. Another fact is that the spread of infection to the blood (bacteraemia) is a later complication of septic abortion and the bacteria present in the blood are transient which is not usually detected because organisms are cleared before the appearance of any clinical finding suggestive of sepsis. Septicemia with negative blood culture may be due to a small number of meticulous microorganisms in the blood stream or may be due to early administration of antimicrobial therapy.

The frequency of blood cultures is also important. It is recommended that two to three blood cultures should be obtained by interval of approximately one hour. Difficulty to collect consecutive blood

samples because of non-compliance of the patients might also contribute to low isolation rate of blood culture.

Regarding the antibiotic susceptibility pattern of predominant bacterial pathogens in the present study, *Staphylococcus aureus* indicated 72-94% sensitivity to amoxycillin-fluocloxacillin, cefoperazone-sulbactam, cefipime and piperacillin-tazobactam. However, they were 63-97% resistant to ceftriazone, amoxycillin-clavulanic acid and penicillin. *Escherichia coli* isolates showed 75-88% sensitivity to cefoperazone-sulbactam, cefotaxime, ceftazidime, piperacillin-tazobactam and cefipime; and 58-96% resistance to ceftriazone, ofloxacin, ciprofloxacin and penicillin.

In a study carried out at NOGH during 2007-8, ceftriazone, ciprofloxacin, amikacin and gentamicin were the effective antibiotics against gram-negative bacilli and clindamycin, oxacillin, ciprofloxacin and gentamicin were effective against gram-positive cocci.<sup>5</sup> Thus, the present study indicated that there was a change in antibiotic susceptibility pattern and emergence of resistant strains to commonly used antibiotics among septic abortion cases in NOGH.

Oxacillin/methicillin resistance implies resistance to all penicillins, cepheims, imipenem and beta-lactam/beta-lactamase inhibitor combinations such as ampicillin-sulbactam, amoxycillin-clavulanic acid, piperacillin-tazobactam, and ticarcillin-clavulanic acid regardless of the *in vitro* test results.<sup>15</sup> In the present study, methicillin-resistant *Staphylococcus aureus* (MRSA) strains were found in 20% (5/25) of *Staphylococcus aureus* isolates.

Extended-spectrum beta-lactamase (ESBL) producing *Escherichia coli* were found in 11.5% (3/26) of *Escherichia coli* isolates in this study. Two patients harbouring ESBL had fatal outcomes. Irrational use of ESBL inducers like ceftazidime in recent years may contribute to emergence and then proliferation of multidrug-resistant



ESBL producing microorganisms especially *Klebsiella spp.* and *Escherichia coli* in hospitals and community. These enzymes hydrolyse the beta lactam ring of 3<sup>rd</sup> generation cephalosporins like ceftriaxone, ceftazidime, cefoperazone, cefotaxime etc. Alternative therapeutic agents like extended-spectrum cephalosporins with beta-lactamase inhibitor combinations i.e., tazobactam, sulbactam, clavulanic acid etc. along with carbapenems are also showing a rise in resistance trend. Incorporation of screening and confirmatory methods for ESBL detection on *Klebsiella* and *E. coli* species have become mandatory for rational use of third generation cephalosporins.

The present study highlighted the predominant bacterial pathogens responsible for septic abortion and emergence of multidrug-resistant bacterial pathogens. The research findings provided the information for development of effective updated antibiotic regime of septic abortion in the respective hospital. As the World Health Organization established a global strategy for containment of antimicrobial resistance, combined efforts by clinicians and microbiologists are needed to implement the framework to prevent emergence of antimicrobial resistance (AMR).

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**Brucellosis: Seroprevalence and the Knowledge, Attitude and Practice (KAP) among Abattoir Workers in Yangon**

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Brucellosis is an important zoonotic disease and an occupational hazard for animal husbandry workers, dairy workers, veterinarians and abattoir workers. A cross-sectional, descriptive study was carried out in Ywarthargyi Slaughter House in Yangon from January to August 2011 to determine the seroprevalence of human brucellosis among abattoirs workers and their knowledge, attitude and practice (KAP) about brucellosis. Face-to-face interviews concerning KAP about brucellosis and collection of blood samples for detection of brucella IgG by ELISA test kit (Organic, France) were carried out. Of 105 abattoir workers, brucella IgG was detected in 4 (3.8%) and only 18 (17%) were aware of brucellosis. Only 10 (9.5%), 13 (12.4%), and 11 (10.5%) knew that infection could be transmitted by drinking of raw milk, by eating of raw meat, and by contact with infected animal, respectively. Regarding the prevention practices, 71 (67.6%) washed their hands with soap thoroughly before and after contact with animals, but only 7 (6.7%) washed their hands with disinfectant. Practices that predispose individuals at risk of infection were also determined. Fifteen (14.3%) engaged in the habit of eating improperly cooked meat and 21 (20%) in the habit of drinking raw milk. This study could help to increase the physician's awareness to the possible existence of the disease in the high-risk human population. It also highlighted a need to improve knowledge and practices of abattoir workers by proper health education.

## INTRODUCTION

Brucellosis is a systemic bacterial disease, the major agents of infection are *Brucella abortus* (relapsing fever) arising from cows and *B. melitensis* (Mediterranean fever) from goats. Other serovars, *B. suis* from pigs and *B. canis* from dogs, have caused isolated incidents of infection.<sup>1</sup> Infections usually occur with acute or insidious onset, characterized by fever, sweats, weakness, malaise and weight loss. The disease may last for several days, months or occasionally for 2 years or more. Humans are generally infected by coming in contact with animals or animal products that are contaminated

with these bacteria and occupational exposure via inhalation of organisms in various work places. It is predominantly an occupational disease of those working with infected animals or their tissues, especially farm workers, veterinarians and abattoir workers.<sup>2</sup>

Brucellosis remains a prevalent disease in humans and animals in many countries around the world, especially those in the Middle East and the Arabian Gulf.<sup>3</sup> In Myanmar, according to the report of the first international conference on emerging zoonoses in Jerusalem, bovine brucellosis and porcine brucellosis cases were found in Myanmar in 1994.<sup>4</sup> Livestock Breeding

and Veterinary Department also reported sporadic cases of bovine brucellosis from certain cattle farms in Myanmar.<sup>5</sup>

There are limited studies of human brucellosis in Myanmar. Our previous study in 2008-9 showed 10.7% brucella IgG seropositive rate among animal handlers and *Brucella* spp. were isolated from 5% of raw milk samples in dairy cattle farms in Yangon Division.<sup>6</sup> The occurrence of bovine brucellosis and serological evidence of brucella infection among the animal handlers in the study areas indicated the need of large-scale study to reveal the magnitude of human brucellosis among occupationally exposed people in Myanmar.

A study reported 3.63% seropositivity of goats from Magway and Mandalay Regions which were subjected to be slaughtered for human consumption. Male sex, those with orchitis and older age (>2 years) goats were found to be significantly associated with seropositivity. Moreover, this study suggested pre-slaughter examination related to brucellosis should be carried out because workers employed in slaughter houses are highly exposed to disease.<sup>7</sup>

Abattoir workers can get the disease through improper handling of contaminated organs and materials. Therefore, the primary preventive programs and health education can be considered as most important ways to prevent the infection among that high-risk population. Educational diagnosis of the occupationally exposed people by assessing knowledge, attitude and practices (KAP) is a useful way in the disease control. To raise the KAP standard of the full spectrum of brucellosis, it is high time to perform this study among vulnerable groups such as laboratory personnel, meat inspectors and slaughter-house workers.<sup>8</sup>

Thus, this study was carried out to determine the seroprevalence of human brucellosis among abattoir workers and their KAP about brucellosis. The research findings may provide necessary information to implement the multi-disciplinary collabo-

ration among the laboratories, research sector, veterinary sector and public health sector to perform successful control measures.

#### *Objectives*

- To detect the brucella IgG antibody by ELISA test in abattoir workers in selected slaughter houses in Yangon
- To identify the knowledge on the nature, risk factors, means of transmission, attitudes and practices (KAP) on brucellosis

## **MATERIALS & METHODS**

#### *Type of study*

A cross-sectional, field- and laboratory-based descriptive study

#### *Study period*

From January to August 2011

#### *Study population*

A total of 105 abattoir workers in Ywarthargyi Slaughter House in Yangon (Yangon City Development Committee) were included in the study. The participants were selected according the following criteria:

- Abattoir workers who are working in the slaughter house for more than 3 months
- Age 18 years and above
- Those who give written informed consent

#### *Data collection*

At the field site (slaughter house), written informed consents were taken from all eligible participants. Face-to-face interviews concerning KAP about brucellosis were conducted on the abattoir workers using pretested structured questionnaires. The questionnaires included:

1. Knowledge about awareness, symptoms, mode of transmission and prevention methods
2. Attitudes towards prevention of the spread of zoonotic diseases

- Practices on preventive measures (hand washing, wearing personal protective equipments) and practices that predispose individuals at risk of infection

#### Detection of brucella IgG antibodies

Two milliliters of venous blood specimens were collected by venepuncture under aseptic condition. The blood samples were sent to Bacteriology Research Division, Department of Medical Research (Lower Myanmar) to perform laboratory tests. From the blood samples, sera were separated by centrifugation. Specimens were kept at  $-20^{\circ}\text{C}$ . Detection of brucella IgG antibodies was done by using Brucella IgG ELISA test kit, Organics, France, according to manufacturer's instruction.

#### Data entry and analysis

EPI DATA software was used for data entry. Range and consistency checks were done. Data analysis was carried out by using SPSS version 13.0 software.

#### Ethical consideration

This study was approved by the Institutional Ethical Review Committee of Medical Research Involving Human Subjects, Department of Medical Research (Lower Myanmar).

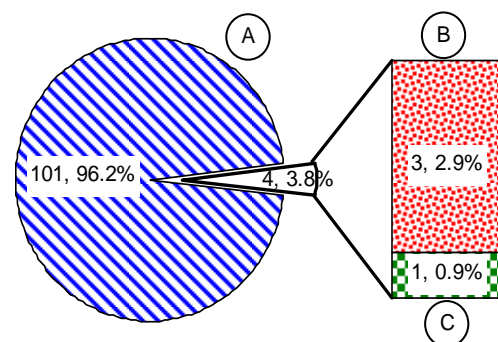
## RESULTS

#### Background characteristics

A total of 105 respondents were recruited. They include 72 workers from cattle slaughter house, 8 workers from goat and sheep slaughter house and 25 from pig slaughter house. All of them were males and their mean age was  $30.1 \pm 10.7$  years (range: 15-59 years). Nine (8.6%) had no formal education, 41 (39%) had primary school level, 35 (33.3%) middle school level, and 20 (19%) high school level of education. Median family income was 150,000 kyats per month.

#### Prevalence of brucella IgG antibody in abattoir workers

A total of 105 blood samples from abattoir workers were tested to detect IgG antibodies to *Brucella* spp. by using brucella IgG ELISA test kit. Brucella IgG was detected in 4 (3.8%) abattoir workers. Three (2.9%) of them were abattoir workers from cattle slaughter house and 1(0.9%) was from goat and sheep slaughter house. None was detected among abattoirs workers from pig slaughter house (Fig. 1).



- A = Seronegative abattoir workers
- B = Seropositive abattoir workers (cattle)
- C = Seropositive abattoir workers (sheep+goat)

Fig. 1. Distribution of brucella IgG seropositive cases among abattoir workers

#### Knowledge, attitudes and practices about brucellosis

Of 105 respondents, 18 (17%) were aware of brucellosis. While 10 (9.5%) knew one symptom (abortion) in cattle, 13 (12.4%) knew at least one symptom in man. Only 10 (9.5%), 13 (12.4%), and 11 (10.5%) knew that infection could be transmitted by drinking of raw milk, by eating of raw meat, and by contact with infected animal, respectively. Prevention by vaccination of animals was known by 12 (11.4%). Fourteen (13.3%) also understood that disease could be prevented by basic hygienic procedures (hand washing) and wearing of protective gloves/masks (Table 1).

Regarding the attitudes, 98 (93.3%) agreed that livestock officer must be informed when animals got sick and died with

Table 1. Knowledge of respondents about brucellosis (n=105)

	Number	Percent
<i>Awareness</i>	18	17
<i>Symptoms of brucellosis</i>		
Abortion in cattle	10	9.5
One symptom in man	13	12.4
<i>Transmission</i>		
Contact with infected animal	11	10.5
Drinking of raw milk	10	9.5
Eating of raw meat	13	12.4
Skin inoculation	11	10.5
<i>Prevention</i>		
Vaccination of animals	12	11.4
Wearing protective gloves/mask	14	13.3
Basic hygienic procedure (hand washing)	14	13.3

unknown cause (more than one case within a day). Ninetysix (91.4%) agreed that it was important to sacrifice the confirmed infectious cattle immediately (Table 2).

Table 2. Attitudes of respondents towards zoonotic diseases (n=105)

	Number	Percent
Agreed that livestock officer must be informed when animals got sick and died with unknown cause	98	93.3
Agreed that it was important to sacrifice the confirmed infectious cattle/pigs immediately	96	91.4

The response to practice questionnaire indicated that 71(67.6%) washed their hands with soap and water thoroughly before and after contact with animals, but only 7(6.7%) washed the hands with disinfectant. Only 5% practiced wearing of gloves or masks while working.

Table 3. Prevention practices and practices that predispose individuals at risk of infection (n=105)

	Number	Percent
<i>Prevention practices</i>		
Washing of hands with soap before and after contact with animals	71	67.6
Washing of hands with disinfectant	7	6.7
Wearing of protective gloves while working	6	5.7
Wearing of protective mask/apron	5	4.7
<i>Practices that predispose individuals at risk of infection</i>		
Eating of improperly cooked meat	15	14.3
Drinking of raw milk	21	20

Practices that predispose individuals at risk of infection were also determined. Fifteen (14.3%) engaged in the habit of eating improperly cooked meat, and 21 (20%) in the habit of drinking raw milk (Table 3).

## DISCUSSION

As *Brucella* spp. is a fastidious bacteria and isolation of the organism is the definitive means of diagnosis, but in practice was difficult due to the early tissue localization and the exacting culture requirements of the organism. Thus, detection of specific antibody of the IgG class to brucella antigen by ELISA is valuable for the diagnosis of recent or previous exposure to Brucella. Evidence of brucella infection through serological examinations has been demonstrated in many countries.<sup>9</sup>

In our study, 3.8% of abattoir workers showed presence of brucella IgG antibodies. This finding is similar to a study in Turkey which showed 2.0 to 12.5% seropositivity among slaughter house workers<sup>10</sup> although it is lower than that of Nigerian study and Saudi Arabian study which showed 63.63% and 35.7% seropositivity, respectively, among similar study populations.<sup>11,12</sup> It is lower than the 10.7% seroprevalence rate of brucellosis among dairy workers in Yangon Division.<sup>6</sup>

Human brucellosis has protean manifestations with complaints ranging from arthralgia, arthritis, myalgia and intermittent fever which get diagnosed as pyrexia of unknown origin (PUO). In the present study, acute brucellosis cases cannot be revealed, however, positive results obtained highlight the existence of disease among high-risk human population and the occupational hazard posed to humans handling the animals.

In Africa, most of the butchers during the screening complained of frequent treatments for malaria without much improvement, while some complained of joint pains and general body weakness.<sup>13</sup> From this

information, it may be suggested that these symptoms might have been as a result of brucellosis which mimics malaria syndrome. The ability of brucella to persist outside mammalian host is relatively high compared with most other non-sporing pathogenic bacteria under suitable condition. Direct and environmental contamination may present hazards through inhalation, ingestion and transmission through mucous membrane or bleached skin.

Thus at the slaughterhouse, personnel who take in charge of opening the carcasses, cutting nodes or udder, or opening the uterus are at higher risk of infection than those who work just processing the meat. There is also a risk from inhalation of aerosols. Collecting the material from an infected uterus may be a major factor in brucellosis contracted inside the abattoir.<sup>11</sup>

In Myanmar, recent studies reported 3.63% seropositivity in goats slaughtered at Mandalay and Magway Division and 16% seropositivity in cattle at Yangon Division.<sup>7, 5</sup> Although the present study cannot correlate with the results of animal brucellosis, 3 seropositive cases were found among abattoir workers who slaughtered cattle, and 1 seropositive case was found in abattoir workers who slaughtered goats and sheep.

In the present study, only 17% of respondents were aware of brucellosis. In a study in Nigeria, awareness about brucellosis was found in 19.6% among occupationally exposed individuals and 33% among butchers.<sup>14</sup> There was also a high level of awareness (76%) among herdsmen in Ghana.<sup>15</sup> Low level of awareness in our study may be related to education level of the participants. More than 80% of them had attained middle and primary school level of education as shown in background characteristics. The present study also confirmed the poor knowledge on symptoms, mode of transmission, and prevention against brucellosis in the study location. But more than 90% of respondents

had a positive attitude towards prevention of the spread of zoonotic diseases.

Ideally, the personal protective equipment of employees in the workplaces with high occupational risk of brucellosis includes appropriate protective clothing (coat, rubber or plastic apron), rubber gloves and boots, eye protection, as well as protection of respiratory exposure. The work clothes should be disinfected after use (heat treatment, chemical disinfectants, etc.) and workers should wash their hands using a disinfectant solution and soap and water with special attention to superficial skin injuries.<sup>16</sup> Good workplace practice, safe work, control and reduction of risks and protective measures should be implemented at any workplace with high occupational risk of infection with *Brucella* spp.<sup>17</sup>

In this study, about two thirds had a good practice of washing hands with soap and water before and after contact with animals despite their poor knowledge on prevention through elementary hygienic procedures such as hand washing (13%). But other prevention practices such as washing hands with disinfectant, and use of protective gloves and masks while working were poor among the study participants. Some of them also appeared to participate in high-risk behaviors such as drinking of raw milk and eating of improperly cooked meat. These habits should be discouraged as it further enhanced the spread of the disease.

### *Conclusion*

This study could help to increase the physician's awareness to the possible existence of the disease in the high-risk human population. It also highlighted a need to improve knowledge and practices of abattoir workers by proper health education in the study area.

### *Recommendation*

- Data on seroprevalence of brucella infection among the study population contribute to develop strategies to increase the awareness of the disease in



the high-risk human population so that necessary precautions and periodic screening of occupationally exposed people can be done.

- KAP data (previously not known) on brucellosis among the study population provide the necessary information to implement the effective preventive programs and health educational programs for control of brucellosis.
- A large-scale epidemiological study on human and animal brucellosis among different study populations in Myanmar should be encouraged to be carried out, thus the magnitude of both human and animal brucellosis can be defined among the study groups.
- Collaboration between veterinary services and public health service is required. There is a great chance of success in prevention and control of zoonotic diseases.

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## Effectiveness of the Local Infiltration of Anti-snake Venom on Experimental Mice Envenomed with Russell's Viper (*Vipera russelii*) Venom

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The immediate administration of intravenous (IV) anti-snake venom (ASV) is an ideal, safe and effective treatment, and intramuscular (IM) ASV administration also has a value as a first aid measure for Russell's viper bite patients. In this study, the effectiveness of different dosages of ASV infiltrated locally at the site of Russell's viper venom (RVV) injection on experimental mice was studied at various time intervals after envenomation, and then compared with those administered intramuscularly and intravenously. The minimum effective doses (ED<sub>50</sub>) of ASV given locally, intramuscularly and intravenously on envenomed mice were found to be 50, 100 and 25 times w/w greater than that of minimal lethal dose (MLD) of RVV, respectively. The obvious neutralizing effect of ASV infiltrated locally was observed if given within an hour after envenomation. It could be suggested that local infiltration of ASV may be an effective first aid measure superior to intramuscular administration in the management of Russell's viper bite envenomation.

### INTRODUCTION

Being a national health problem in Myanmar, a vast amount of study and research on Russell's viper (*Vipera russelii*) bite envenomation have been carried out in the Department of Medical Research (Lower Myanmar) (DMR-LM) for many decades in order to reduce morbidity and mortality associated with snake bite. Studies on the pattern of <sup>125</sup>I labeled Russell's viper venom (RVV) distribution in mice revealed that muscle of the injection site was one of the major organs for accumulation of RVV and there was also a slow declining level of venom at the site of injection with the time elapsed.<sup>1</sup>

Therefore, it is suggested that early removal of venom from the site of bite, by any means, is necessary to reduce the amount of venom available for further spreading into the body compartments and to prevent rapid envenomation. Nowadays, local incision of muscle at the site of bite for removal of the injected venom is totally discouraged since

it produces undesirable tissue damage and uncontrolled bleeding in victims with disseminated intravascular coagulation (DIC) after bite.<sup>2</sup> Therefore, an alternative measure i.e., local infiltration of anti-snake venom (ASV) into the site of bite or envenomation may be useful in neutralization of injected venom.

Therefore, this study was intended to determine the effectiveness of ASV infiltrated locally into the site of envenomation with different dosages at various time intervals after injection of RVV. Then, its efficacy was evaluated and compared with those of intramuscular (IM) and intravenous (IV) ASV administrations.

### MATERIALS AND METHODS

Adult albino mice, the Institute of Cancer Research (ICR) strain of both sexes, weighing 20±1 g were supplied by Laboratory Animal Services Division, DMR-LM. Russell's viper venom (RVV) as a desiccated form and nonspecific enzyme refined



anti-Russell's viper venom (ASV) as a freeze dried form were purchased from Myanmar Pharmaceutical Factory (MPF), Yangon.

#### *Experimental design*

Intramuscular (IM) lethal doses (LDs) of RVV on mice were first determined and calculated. Then, three groups of mice, envenomed intramuscularly into hind leg with minimum lethal dose (MLD) i.e., LD<sub>100</sub> of RVV, were given various neutralizing doses of ASV (greater than MLD of RVV) immediately via three routes; namely local infiltration into the same site of RVV injection, IM (other hind leg) and IV (tail vein), respectively.

Then the same experiment was repeated. This time, the neutralizing doses were given at various time intervals, i.e., 0, 0.25, 0.5, 1, 2, 4 and 8 hours after envenomation. The numbers of mice which survived after 24 hours were then determined.

#### *Determination of IM lethal doses (LDs) of RVV on mice*

A total of 30 mice were divided into 5 groups each consisting of 6 mice. RVV dissolved in normal saline was injected intramuscularly into the hind leg muscle with different amounts ranging from 0.625 to 10 µg/g body weight (BW). The mice of control group received normal saline (N/S) only. The number of mice which died within 24 hours were recorded.

#### *Study on the effectiveness of different routes of administration of ASV on envenomed mice immediately after envenomation*

A total of 48 mice equally divided into 8 groups were tested. Immediately after injection with the minimum lethal dose (MLD) of RVV into hind leg muscle, the various neutralizing doses of ASV ranging from 7.9 g to 500 µg/BW (i.e., 1.56 to 100 times w/w greater than MLD of RVV) were locally infiltrated into the same site of the injecting RVV. Control mice were given normal saline instead of ASV after envenomation. The number of mice which survived after 24 hours were observed.

The same procedure as mentioned above was carried out on envenomed mice, immediately followed by the various neutralizing doses of ASV administered intramuscularly into another hind leg as well as intravenously through the tail vein of the envenomed mice in the two separate experiments.

Then, the effective doses (EDs) of ASV in different routes of administration on envenomed mice immediately after envenomation were calculated by using Spearman-Karber method.<sup>3</sup>

#### *Study on the effectiveness of different routes of administration of ASV on envenomed mice at various time intervals after envenomation*

A total of 64 mice were equally divided into 8 groups. They were envenomed with the MLD of RVV, followed by the local infiltration of ASV using ED<sub>100</sub> of IV administration to the same site of injecting RVV at different time intervals ( i.e., 0, 0.25, 0.5, 1, 2, 4 and 8 hours) after envenomation. No ASV was followed in envenomed mice of the control group. The number of survivals was noted after 24 hours. Another two separate experiments were carried out for the IM and IV ASV administration at various time intervals after envenomation.

## RESULTS

The IM median lethal dose (LD<sub>50</sub>) and minimum lethal dose (MLD) i.e., LD<sub>100</sub> of RVV on mice were found to be 1.7 µg/g BW and 5 µg/g BW, respectively, calculated by Spearman-Karber method<sup>3</sup> (Table 1).

Table 1. Estimation of IM lethal doses (LDs) of Russell's viper venom (RVV) on mice

Doses of RVV given (µg/g) BW	Lethality within 24 hours	
	No.	(%)
10	6/6	(100)
5	6/6	(100)
2.5	3/6	(50)
1.25	2/6	(33.38)
0.625	1/6	(16.7)
Control (N/S)	0/6	(0)

N/S= Normal saline      Calculated LD<sub>50</sub>=1.7 µg/g BW  
 LD<sub>90</sub> = 4.87 µg/g BW  
 LD<sub>100</sub> = 5.0 µg/g BW

Table 2. Determination of the effectiveness of anti-snake venom in different routes administered immediately after envenomation

ASV:RVV* (w/w )	Neutralizing dosages of ASV ( $\mu\text{g/g}$ BW)	Survivals after 24 hrs		
		Local No. (%)	IM No. (%)	IV No. (%)
100:1	500	6/6(100)	6/6(100)	6/6(100)
50:1	250	6/6(100)	5/6(83)	6/6(100)
25:1	125	4/6(66.7)	3/6(50)	6/6(100)
12.5:1	62.5	3/6(50)	2/6(33.3)	5/6(83.3)
6.3:1	31.3	3/6(50)	2/6(33.3)	3/6(50)
3.1:1	15.7	2/6(33.3)	1/6(16.7)	2/6(33.3)
1.6:1	7.9	1/6(16.7)	0/6(0)	1/6(16.7)
0.0:1	Control	0/6(0)	0/6(0)	0/6(0)

\*Envenoming dose of RVV=5  $\mu\text{g/g}$  BW (i.e., LD<sub>100</sub>)

The data on determination of the effectiveness of ASV given by different routes administered immediately after envenomation of mice with MLD are shown in Table 2. It was found that the same doses of ASV i.e., 25 times w/w greater than that RVV administered locally, intramuscularly and intravenously could apparently save 66.7%, 50% and 100% of envenomed mice, respectively.

Table 3. Comparative efficacy of calculated effective doses (EDs) of ASV in different routes of administration on envenomed mice

	Dosages of ASV ( $\mu\text{g/g}$ BW)		
	Local	IM	IV
ED <sub>50</sub>	49.6(9.9)	78.7(15.7)	24.8(4.9)
ED <sub>90</sub>	205(41)	385(77)	95(19)
ED <sub>100</sub>	250(50)	500(100)	125(25)

Envenoming dose of RVV=5  $\mu\text{g/g}$  BW (i.e., LD<sub>100</sub>)  
Values in parentheses indicate the ratio of dosages of ASV w/w greater than that of the RVV injected

Table 3 summarizes the comparative efficacy of local, IM and IV administrations of ASV on envenomed mice, mentioned as calculated ED<sub>50</sub>, ED<sub>90</sub> and ED<sub>100</sub>. It was observed that although the effectiveness of the local infiltration of ASV was inferior to that of IV route, it was remarkably superior to IM route if given immediately after envenomation. Effectiveness of the various routes of ASV administration at different time intervals on mice envenomed with MLD of RVV is shown in Table 4.

Table 4. Effectiveness of the various routes of ASV administered on envenomed mice at different time intervals after envenomation

Times of initiating ASV after envenomation (hours)	Survivals of mice after 24 hours		
	Local No. (%)	IM No. (%)	IV No. (%)
0	5/8(62.5)	4/8(50)	8/8(100)
0.25	5/8(62.5)	3/8(37.5)	7/8(87.5)
0.50	5/8(62.5)	3/8(37.5)	7/8(87.5)
1.0	3/8(37.5)	2/8(25)	5/8(62.5)
2.0	0/8(0)	1/8(12.5)	4/8(50)
4.0	0/8(0)	0/8(0)	2/8(25)
8.0	0/8(0)	0/8(0)	0/8(0)
RVV alone	0/8(0)	0/8(0)	0/8(0)

Envenoming dose of RVV=5  $\mu\text{g/g}$  BW (i.e., LD<sub>100</sub>)  
Neutralizing dose of ASV=125  $\mu\text{g/g}$  BW (i.e., ED<sub>100</sub> of IV)

It was evident that IV route of ASV administration was found to be the most effective treatment up to 4 hours after envenomation whereas local infiltration of ASV given within an hour produced a remarkable efficacy over the IM route of administration. However, local infiltration of ASV was found to be unable to neutralize the effect of RVV when administered 1 hour after envenomation at which both IM and IV ASV administration showed some extent of neutralizing efficacy.

## DISCUSSION

In Myanmar, an immediate administration of IV ASV is an ideal, safe and effective treatment for Russell's viper bite patients, and IM ASV administration is also of value as a first aid measure in the field where IV route is not possible.<sup>4, 5</sup> In spite of utilizing various effective measures in clinical practice, morbidity and mortality associated with Russell's viper bite is unacceptably high in our country. Therefore, simple and easiest way of administration i.e., local infiltration of ASV, has been taken into consideration as an alternative first aid measure, for management of snake bite victims prior to hospitalization.

In this experimental study, local infiltration of ASV in dosage of 50 times w/w greater than that of RVV into the muscle at

the injection site produced 100% survivals and 25 times w/w greater than that of RVV revealed about 66.7% survivals in envenomed mice whereas 83% and 50% survivals in IM administration, respectively (Table 2). Therefore, it is to be noted that ASV local infiltration given immediately after envenomation showed a more favorable effect than that of IM ASV given in same dosage if the same amount of venom had been injected into mice.

Regarding the various time intervals between envenomation and initiation of ASV, the efficacy of ASV given in all routes of administration was found to be reduced with increasing time intervals (Table 4). It is to be noted that local infiltration of ASV within half an hour post envenomation in mice revealed a remarkable efficacy (62.5% survival) compared to that of IM route (37.5%). Therefore, an efficacy of local ASV given within an hour may be superior to that of IM route. However, local infiltration of ASV was found to be no longer effective if given after an hour post envenomation. Likewise, IM and IV administration of ASV were found to be unable to neutralize the effects of RVV when administered 2 and 4 hours after envenomation, respectively (Table 4).

It is also interesting to note that all envenomed mice followed by IM and IV ASV administration showed acute myonecrotic change at the sites of RVV injection whereas those followed by local infiltration of ASV were found to have no apparent local reactions. It may be due to the prompt neutralization of injected venom by ASV directly infiltrated into the site of envenomation.

This finding is somewhat contrary to the facts mentioned in the WHO SEARO Guidelines for the clinical management of snake bite in Southeast Asian Region in which it is noted that local infiltration may

increase intracompartmental pressure and the risk of hematoma formation.<sup>6</sup>

### Conclusion

This preliminary study showed that local infiltration of ASV in an adequate dose administered to the site of injection within an hour after envenomation is more effective first aid measure compared to that of IM administration in the experimentally envenomed mice. However, the findings of research work, conducted on animal models, cannot be directly applied to human beings. Therefore, further attempts need to be carried out for studying safety and efficacy of local ASV infiltration before being applied on human snake bite victims.

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