

VIROLOGY RESEARCH DIVISION

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During this period (2015) the Virology Research Division was involved in three main research areas: namely viral diarrhoea, arbovirology and viruses causing acute respiratory infections. The research projects were mostly involved in disease surveillance of viral infections for timely prevention of disease outbreaks. Also, some of the studies were aimed to monitor the emergence of new viral strains or subtypes to provide base-line data for the formulation of effective candidate vaccines and for elucidating the contribution of viral genetics to the changing patterns of disease.

RESEARCH PROJECTS

1. COMMUNICABLE DISEASES

1.1. DIARRHOEA/DYSENTERY

1.1.1. Surveillance of rotavirus diarrhoea in Yangon Children Hospital in 2015

Rotavirus gastroenteritis (RVGE) is the leading cause of severe diarrhoea affecting young children worldwide. To reduce the burden of RVGE, prevention by vaccination is the most effective strategy. In consideration of rotavirus vaccine (RV), not only epidemiological information likes prevalence, age and gender distribution, seasonal variation and severity of disease is required, but also identification of currently circulating genotypes are crucial for evaluation of the effectiveness of a vaccine. Therefore, this study was carried out to determine the epidemiology of rotavirus diarrhoea and genotype distribution among less than 5 years old children attending the three medical wards of Yangon Children Hospital. Stool specimens not less than 3 ml were collected from diarrhoeic children as soon as possible after hospital admission and transported daily to the laboratory in the Virology Research Division. From January to December 2015, a total of 868 stool samples were collected and clinical features were recorded in the Case Report Form. All the samples collected were tested for the presence of rotavirus antigen by a commercial enzyme immunoassay kit (ProSpecT™ Rotavirus from OXOID, UK). Among 868 samples tested, rotavirus was detected in 456 (53%) of cases. Rotavirus diarrhoea was most prevalent in the 6-11 months age group and

during the cooler, drier months of the year (January and February). The majority (73%) of the rotavirus positive cases were severe diarrhoea according to the Vesikari clinical severity scoring system. From rotavirus positive samples, 125 samples were selected and analyzed by RT-PCR for genotyping. Regarding G genotype, 102 (81.6%) was found to be G9 genotype, 1 (0.8%) was G1 genotype, 3 (2.4%) was G2 genotype and 16 (12.8%) was untypable and mixed genotypes was 3 (2.4%). As for P typing, 73 (58.4%) was P[8], 6 (4.8%) was P[6], 1 (0.8%) was P[4], 40 (32%) was untypable and mixed genotypes was 5 (4%). The most common combination was G9P[8] followed by G9UT and UTP[8]. Continuous surveillance of RVGE is an important platform which will provide vital information for considering the target population and timing of vaccination plan in the future by policy makers and vaccine programmers and will also be enable monitoring the effectiveness and long-term impact of vaccination on rotavirus epidemiology and on circulating rotavirus strains.

Percent distribution of G genotype by year (2011-2015)

	G1	G2	G3	G9	G8	G12	G1+ G12	G2+ G9	G9+ G12	G2+ G10	G2+ G12	G1+ G9	Unty- pable
2011	5.0	3.0	0.0	1.0	0	87.0	2.0	0.0	0.0	0	0	0.0	2.0
2012	24.0	13.0	1.0	7.0	0	42.0	1.0	0.0	2.0	0	0	0.0	10.0
2013	42.0	29.0	0.0	22.0	0	1.0	0.0	0.0	0.0	0	0	0.0	6.0
2014	18.0	7.0	0.0	63.0	0	0.0	0.0	0.0	0.0	0	0	0.0	12.0
2015	0.8	2.4	0.0	81.6	0	0.0	0.0	1.6	0.0	0	0	0.8	12.8

Percent distribution of P genotypes by year (2011-2015)

	P[4]	P[6]	P[8]	P[9]	P[4]+ P[6]	P[4]+ P[8]	P[6]+ P[8]	P[9]+ P[8]	Unty- pable
2011	3.0	22.0	64.0	0.0	0	0.0	2.0	0.0	9.0
2012	13.0	15.0	54.0	1.0	0	0.0	5.0	1.0	12.0
2013	29.0	6.0	54.0	0.0	0	0.0	0.0	0.0	11.0
2014	2.0	2.0	70.0	0.0	0	2.0	0.0	0.0	24.0
2015	0.8	4.8	58.4	0.0	0	0.8	3.2	0.0	32.0

1.1.2. Hospital-based surveillance of intussusception among children in Yangon (2015)

The rotavirus vaccine “RotaShield” was licensed in 1998 and withdrawn in 1999 because of the demonstrable association between receipt of this vaccine and intussusceptions. Since then, the important first step for making decisions regarding conducting trials of introducing rotavirus vaccine in developing countries is to understand the epidemiology and baseline risk of intussusception in these settings. With this concept, surveillance for intussusception among children less than 2 years of age admitted to the surgical wards of Yangon Children Hospital was conducted. In the year 2015, among 5395 less than two years old children admitted to the surgical wards of Yangon Children Hospital, 2168 were presented with acute abdomen and among them 73 cases were diagnosed as intussusception. Age range of patients was from 4 to 12 months and presented with bloody mucus diarrhoea, vomiting and fever. Out of 73 intussusception cases, stool samples could be collected from 12 cases and tested for the presence of rotavirus by a commercial ELISA kit (ProSpecT™ Rotavirus, Oxoid, UK). Rotavirus was not detected in all cases. This data will provide baseline data for investigators and programmers in balancing the risk and benefit in post vaccination period in the future.

Intussusception cases admitted to YCH from 2011 to 2015

	2011	2012	2013	2014	2015
Number of <2 year admission	1822	1711	679	3146	5396
Number of Intussusception cases	14	44	36	32	73
Number of stool collected	3	9	-	7	12
ROTA Elisa test	Negative	Negative	-	Negative	Negative

1.1.3. Effectiveness of oral Immunoglobulin Y supplemented infant formulae treatment for rotavirus diarrhoea in 6 months to 59 months old children admitted to Yangon Children Hospital

This study aimed to evaluate the effect of oral Immunoglobulin Y supplemented infant formulae against rotavirus infection in a clinical trial in paediatric patients hospitalized with rotavirus diarrhoea. IgY is an immunoglobulin produced in the egg yolk of immunized chickens. Brand name of anti-HRV IgY is called "Ovalgen RV". Infant formula supplemented with and without Ovalgen RV was dispensed into sachets and labeled as "A" and "B". The total duration of the study was 3 months (from January to March, 2015). This study was equally randomized double-blind controlled clinical trial. A total of 100 dehydrated and rotavirus-positive children admitted to YCH were randomized into Test group (Group A treated by "Sachet A") and Control group (Group B treated by "Sachet B"). Of these, only 94 children (47 children in each group) completely participated. All patients received standard supportive therapy for diarrhoea. Two sachets of A and B were given orally, every 6 hours to test group and control group respectively. From all subjects, approximately 3 mls of stool was collected with a sterile screw-capped container, daily for 5 consecutive days. Each day, one part of the stool sample was analyzed by Dipstick "Eiken" ROTA kit to detect the presence of rotavirus infection. The rest of the stool samples were stored at -20° C at the Virology Research Division until ELISA test and genotyping of rotaviruses was done. The patients were monitored for daily diarrhoea and vomiting frequency, volume of oral rehydration fluid (ORF) and intravenous fluid (IVF) intake, and rotavirus shedding duration. Bacterial analysis of stool samples was performed on all patients in groups A and B on days 1 and 5 during the treatment.

Statistically significant difference between the two groups was found particularly in terms of daily rotavirus shedding frequency on day 2 ($p=0.04$) and reduction in co-infection rate ($p=0.002$). The most frequent co-infection combinations were rotavirus-*E. coli*. Compared to Control group, the Test group showed faster mean duration of virus clearance from stool ($p=0.17$) and reduction of ORF intake ($p=0.25$) although the difference in effect was not statistically significant. Moreover, Ovalgen RV was found to be safe to administer as adverse effects and unusual responses were not seen in any of the participants. In overall, Ovalgen RV supplemented infant formula appears to be a promising, safe and adjunct to rehydration therapy in management of acute rotavirus diarrhoea in pediatric patients.

Parameters	Test Group		Control Group		p value
Bacterial co-infection rate before and after taking IgY	38% (Day 1)	11% (Day 5)	26% (Day 1)	13% (Day 5)	p=0.002*
Daily rotavirus shedding frequency	100% (Day 1)	80% (Day 2)	100% (Day 1)	94% (Day 2)	p=0.04*
Mean duration of rotavirus shedding in stool	3.83 days		4.09 days		p=0.17
Mean ORF intake on day	3193 ml		3400 ml		p=0.25

1.2. DENGUE HAEMORRHAGIC FEVER

1.2.1. Dengue virus serotypes among dengue haemorrhagic fever patients in Yangon Children Hospital (2015)

This study was carried out to determine the prevailing dengue virus serotypes in children with dengue infection admitted to the medical wards of Yangon Children Hospital from January to December 2015. The sera samples were collected from clinically diagnosed dengue patients and serological confirmation was done by Immunochromatographic test (SD BIOLINE Dengue Duo NS1 Ag and IgG/IgM). Out of 878 serum samples tested, 531 (60.4%) were serologically confirmed dengue infection. Among them, 229 (43%) was found to be secondary dengue infection, 67 (13%) was primary infection and the rest 235 (44%) was NS1Ag alone positive and may be early primary or secondary infection. Some positive samples with early fever days (<5 days) and some acute seronegative samples were subjected to virus isolation by tissue culture in C6/36 mosquito cell lines with 24 well culture plates and further typed by indirect Immunofluorescent Antibody Technique with serotype specific monoclonal antibodies to the four dengue viruses. Of the 103 samples subjected to isolation, dengue virus was isolated from 20 samples, accounting for 19% isolation rate. Among the isolated viruses, 14 samples (70%) were DENV1, 2 (10%) were DENV2, 1 (5%) was DENV3 and 3 (15%) were mixed infection of DENV1+2 and DENV1+4. Thirteen isolates (65%) were from DHF I, 2 isolates (10%) was from DHF II cases, 3 isolates (15%) was from DHF III and 2 (10%) was from DHF IV cases. Out of 3 DHF III cases, two cases were caused by DENV2 and 1 case by DENV1. All DHF IV cases were caused by DENV1. Continuous and comprehensive virological surveillance is crucial for identification of currently circulating strains and timely detection of new viral strains, which is essential for the prediction of occurrence of outbreaks and severity of the disease and provides beneficial information for preventive and control interventions.

Distribution of Dengue virus serotypes from 2011-2015

Year	Total isolates / RT-PCR	DENV-1	DENV-2	DENV-3	DENV-4	Mixed
2011	3	3	0	0	0	0
2012	7	2	1	0	3	1 (D1+4)
2013	36	34	0	0	2	0
2014	30	14	5	2	9	0
2015	20	14	2	1	0	3

1.2.2. Molecular detection of dengue virus serotypes affecting adult population admitted to the medical wards of Yangon General Hospital during 2015 dengue outbreak

Dengue is typically acknowledged to be a childhood disease and important cause of hospitalization, however, a shift in affected age of dengue infection to older age group was reported in Thailand, Singapore, Indonesia and Bangladesh. In the 2015 dengue outbreak in Myanmar, not only children but also adults were hospitalized even with bleeding manifestation. As the infecting dengue serotype has great influence on the severity of the disease and there is knowledge gap concerning adult dengue in Myanmar population, this study was carried out with the aim to identify dengue serotypes responsible for the emergence of adult dengue cases. A total of 33 blood samples were collected from serologically confirmed dengue cases (tested by SD BIOLINE Dengue Duo NS1 Ag and IgG/IgM)

admitted to the medical wards of Yangon General Hospital between July and September 2015. Among them, 11 (33.3%) was primary dengue infection and 22 (66.7%) was secondary dengue infection. The mean age was 20.79 ± 7.56 years, the youngest and oldest being 13 years and 48 years respectively. The male patients accounted for 69.7% (23/33) and female patients for 30.3% (10/33). Regarding severity of disease, 10 cases (30%) were DHF I, 20 cases (60%) were DHF II and 1 case each (3.3%) was DF, DHF III and DSS respectively. Both DHF III and DSS cases (severe dengue) were secondary infection. RNA was extracted from the sera using QIAamp Viral RNA extraction columns (Qiagen) and serotype was identified by multiplex RT-PCR. Out of 33 samples analyzed, dengue virus was detected in 6 samples (1 in DF, 2 in DHF I and 3 in DHF II) and all were found to be DENV-1 which has the predominant serotype since 2001. These data will contribute as an important piece of bridge that will fill the knowledge gap about epidemiology and serotype information about adult dengue in Myanmar population.

1.3. ACUTE RESPIRATORY INFECTIONS

1.3.1. Rubella Virus

1.3.1.1. Detection of measles, rubella and parvovirus B19 in children with fever and rash attending Out Patient Department of Yangon Children Hospital

Fever and rash is a common presentation of childhood viral infections. Although there are many etiologies responsible for fever and rash, measles and rubella are frequent causative agents and represent public health concerns in Myanmar. In addition, parvovirus B19 is a common viral infection of childhood that has not been studied in Myanmar. The aim of this study was to identify measles, rubella and parvovirus B19 infection in children with fever and rash admitted to medical wards of Yangon Children Hospital. A total of 176 oral fluid and blood samples were collected from 2 months to 12 years old children presenting with fever and rash at YCH during 2013-2014. The samples were collected within 7 days of illness to detect nucleic acid and IgM of measles virus, rubella virus and parvovirus B19. Extraction of RNA was done on 176 oral fluid samples and then analyzed by nested real time polymerase chain reaction using primers from Measles and Rubella Reference Laboratory from Institute of Immunology, Luxembourg to detect measles and rubella virus. After the detection step, positive samples were confirmed again by detection of E1 gene and N450 gene for rubella virus and measles virus respectively. Out of 176 samples, 14 (7.95%) were found to be positive for N450 gene of measles virus and 11 (6.25%) were positive for E1 gene of rubella virus. For parvovirus B19, nested conventional PCR was done on 176 serum samples and 4 (2.27%) were positive for parvovirus B19. IgM ELISA tests for those viruses were also done on 176 serum samples and IgM were positive in 12 samples (6.8%) for measles, 15 (8.5%) for rubella, and 5(2.8%) for parvovirus B19. There was no history of MR or MMR vaccination among the study population .This is the first molecular study in Myanmar for detecting measles and rubella viruses from oral fluid and also determining the presence of parvovirus B19 in Myanmar. Findings from this study contribute the baseline virologic data for implementation of measles, rubella and parvovirus infections control strategy.

1.3.2. Influenza Virus

1.3.2.1. Molecular characterization of influenza strains circulating in Yangon Children Hospital (2015)

A cross-sectional descriptive study was conducted at Out Patient Department (OPD) of Yangon Children Hospital (YCH) with the aim to determine the predominant subtype of influenza virus among children attending YCH in 2015. A total of 67 children with influenza-like illness (ILI) attending YCH from January to August 2015 were recruited and throat swabs and nasal swabs were taken from them. Viral RNA was extracted from the specimens by RNA extraction kits (QIAamp® Viral RNA Mini Assay). Matrix genes of influenza A virus and influenza B virus were detected by reverse transcriptase-polymerase chain reaction (RT-PCR) using Qiagen one step RT-PCR kit. Influenza A virus positive samples proceeded to subtyping by multiplex RT-PCR detecting haemagglutinin gene of influenza A (H1N1) pdm09 virus, seasonal influenza A (H1N1) virus, seasonal influenza A (H3N2) virus and avian influenza A (H5N1). Of 67 ILI cases, influenza A virus was detected in 6 cases (8.9%) and no case of influenza B virus was detected. Female to male ratio was 5:1. Majority of influenza virus positive cases (50%) were between 5 to 9 years of age. Five out of six positive cases were detected in July and one case in August. Of influenza A virus positive cases, 4 cases (66.7%) revealed HA gene of influenza A (H3N2) virus and 2 cases (33.3%) revealed that of influenza A (H1N1) pdm09 virus. So, the predominant subtype of influenza virus among children attending YCH in 2015 was influenza A (H3N2) virus which was also prevailed among children attending YCH in 2013 and 2014.

Prevalence of pathogenic subtypes of influenza A virus among children attending YCH (2013-2015)

	2013	2014	2015
Total ILI cases	100	98	67
Cases of influenza B virus	0/100 (0%)	0/98 (0.0%)	0/67 (0.0%)
Cases of influenza A virus	6/100 (6%)	7/98 (7.1%)	6/67 (8.9%)
• Cases of influenza A (H1N1) virus	0/6 (0.0%)	0/7 (0.0%)	0/6 (0.0%)
• Cases of influenza A (H1N1)pdm09 virus	1/6 (16.7%)	1/7 (14.3%)	2/6 (33.3%)
• Cases of influenza A (H3N2) virus	5/6 (83.3%)	6/7 (85.7%)	4/6 (66.7%)
• Cases of influenza A (H5N1) virus	0/6 (0.0%)	0/7 (0.0%)	0/6 (0.0%)

1.3.3. Other Respiratory Viruses

1.3.3.1 Bacterial, viral and atypical pathogens associated with acute respiratory infections and their clinical characteristics among children admitted to Yangon Children Hospital: Virology Findings

Acute Respiratory Infections are the major causes of morbidity during early childhood. The aim of this study is to establish the multiplex PCR assay to detect respiratory pathogens in under 5 children attending Yangon Children Hospital. This study was carried out in collaboration with Nagasaki University, Japan. A total of 390 nasopharyngeal swab specimens were collected from under 5 children with ARI attending medical wards of Yangon Children Hospital from February 2014 to August 2015. Regarding viral aetiology, 13

respiratory viruses such as human rhinovirus, human adenovirus, respiratory syncytial virus (RSV), influenza A virus, influenza B virus, parainfluenza virus 1, parainfluenza virus 2, parainfluenza virus 3, parainfluenza virus 4, human metapneumovirus, human coronavirus 229E, human coronavirus OC43 and bocavirus were detected by 4 assays of multiplex PCR. Respiratory viruses were detected in 157 cases (40.26%). Among them, human rhinovirus was found to be most prevalent accounting for 18.5% (n=72) of the cases, which was followed by RSV (30/390; 7.7%), adenovirus (17/390; 4.4%), parainfluenza virus 3 (17/390; 4.4%), influenza A virus (13/390; 3.3%), bocavirus (8/390; 2%), human coronavirus (8/390; 2%), human metapneumovirus (3/390, 0.8%), parainfluenza virus 1 (3/390; 0.8%), parainfluenza virus 2 (3/390; 0.8%) and parainfluenza virus 4 (2/390; 0.5%). There was no case of influenza B virus infection among the ARI cases. Multiple viral infections were also found in the study that were responsible for 7.6% (n=28) of the cases. Rhinovirus infection cases co-infected with other respiratory viruses were commonly detected that accounted for 71.4% (n=20) of multiple infection cases. Three out of 28 (10.7%) multiple infection cases showed triple infection of rhinovirus and two other respiratory viruses.

SERVICES PROVIDED

ACADEMIC

Sr.	Name	Course	Responsibility
1.	Dr. Mo Mo Win	MMedSc(Microbiology)	Teaching
		MPH	Teaching
		MMedTech (Med Lab Tech)	Teaching
		BMedTech (Med Lab Tech)	Teaching
2.	Daw Khin Mar Aye	MMedSc (Microbiology)	Teaching
3.	Dr. Htin Lin	MMedSc (Microbiology)	Teaching
4.	Dr. Nila Zaw	MMedSc (Microbiology)	Teaching

LABORATORY

Sr.	Subject	Tested samples
1.	Performing platelet counts for patients admitted to YCH with suspected DHF and other bleeding disorders.	1299 samples
2.	Performing Western Blot tests for confirmation of HIV-1 antibody	62 samples

VIROLOGY RESEARCH DIVISION (POL)

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	...	Daw Thida Aung BSc(Chemistry)(Meikhtila University)
	...	Daw Yin Yin Khine BSc(Physics)(Meikhtila University)
Research Assistant (4)	...	Daw Khin Moh Moh Win

Virology Research Division has been actively engaged in conducting a number of research projects mainly in an area of communicable diseases. The division collaborates with other Research Divisions of DMR (POLB) as well as other institutions to carry out research projects.

RESEARCH PROJECTS

1. COMMUNICABLE DISEASES

1.1 DIARRHOEAL DISEASES

1.1.1 Antimicrobial resistance for acute watery diarrhoea management among patients admitted in hospitals of Mandalay city
(Please refer to the Annual Report of Bacteriology Research Division)

1.2 VIRAL INFECTION

1.2.1 Isolation of Flavivirus from Culex mosquitoes collected in Myanmar, 2014

Myanmar, a country without existing immunization programme against the flavivirus Japanese encephalitis virus (JEV), has a high incidence of Japanese encephalitis. It also has high incidence of infection due to dengue virus (DENV), another flavivirus. Surveillance for JE was carried out in a hospital in 2013 and for dengue at two study areas (Upper Myanmar and lower Myanmar) during the last seasonal outbreak in 2015 and the collected clinical samples were subjected to serological tests and viral isolation with molecular analysis. Among 123 cerebrospinal fluid samples from patients with acute encephalitis syndrome in 2013, (3.3%) were positive for IgM antibody against JEV and confirmed further for JE by neutralization tests. Among 330 acute phase serum samples from clinically diagnosed patients in two study areas, (31.5%) yielded DENV with two samples having co-infection. There were (76) DENV-1, 24 DENV-2, (1) DENV-3 genotype I and (5) DENV-4 genotype I. Field-based flavivirus surveillance from mosquitoes was conducted in 2014 and 19 virus strains of mosquito-specific flavivirus were isolated in this study. In conclusion, both JE and DENV were circulating in Myanmar and DENV-1 is predominant during 2015 seasonal outbreak in Myanmar.

1.3 TUBERCULOSIS

1.3.1 Detection of *Mycobacterium tuberculosis* and its resistance to INH and Rifampicin by Microscopic Observation Drug Susceptibility (MODS) assay in adult patients with suspected pulmonary tuberculosis

The MODS assay is a simple, rapid and liquid culture-based diagnostic method. The clinical and laboratory-based descriptive study was carried out in 2014-2015. A total of 135 TB suspected patients were included after obtaining written informed consent. This study aimed to detect *Mycobacterium tuberculosis* and its resistance to isoniazid and rifampicin in sputum smear positive and negative pulmonary TB, Gene Xpert proved TB with Rifampicin resistance TB, positive and negative cases by MODS assay and to determine its sensitivity and specificity. MODS assay showed the culture positivity in 80% of sputum smear positive and 63.6% of negative cases done by Ziehl-Neelsen stain, 79.2% sputum smear positive and 56.5% negative cases done by fluorescence microscopy. Ninety percent of Gene Xpert detected with RR positive and 88.1% of RR negative cases showed positive culture on MODS assay at day 9. Among 62 Gene Xpert detected cases, 19.4% were culture positive with INH resistance on MODS assay. MODS assay expressed culture positive, RR positive on 85% of Gene Xpert detected, RR positive cases and culture positive, RR negative on 95.2% of Gene Xpert detected, RR negative cases. Therefore MODS assay can aid the diagnosis of mono and multi resistant tuberculosis.

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

Globally, tuberculosis (TB) still remains a major public health problem. Global efforts for TB control, especially in resource limited settings, are being challenged by the lack of rapid, reliable and inexpensive techniques for the detection of *Mycobacterium tuberculosis*. MODS is a manual liquid culture technique that uses an inverted light microscope to detect microscopic colonies of *M. tuberculosis* grown in culture medium in sealed multiwell plastic plates. This study has three distinct comparisons: (i) comparison of the sensitivity of detection of mycobacterial growth by MODS with that by conventional culture on Lowenstein-Jensen (LJ) medium; (ii) comparison of the time to culture positivity of sputum specimens culture positive by both MODS and LJ culture; and (iii) evaluation of the concordance of the susceptibilities obtained by phenotypic drug susceptibility testing of clinical samples by MODS and by genotypic drug susceptibility testing by line probe assay. The study population was selected from the patients with suspected pulmonary tuberculosis attending Tuberculosis Centre, Upper Myanmar. A total of 98 cases including 80 sputum smear positive cases and 20 sputum smear negative cases were included in this study. Thirty eight cases were culture positive by MODS assay whereas 25 cases were culture positive by solid culture. Among culture positive cases, 26 cases were rifampicin resistance by MODS assay and 4 cases were rifampicin resistance by line probe assay. Eleven cases were isoniazid resistance by MODS assay and 7 cases were isoniazid resistance by line probe assay.