Surveillance of Drug Resistance in Recurrent Leprosy cases using Mouse Foot Pad (MFP) Assay

Krishus Nepal[1*], Nischal Pokhrel[1], Bishwanath Acharya[1], Divya RSJB Rana[1], Binod Aryal[1], Kancha Shrestha[2], Suwash Baral[2], Reejana Shrestha[2], Deanna Hagge[1], Mahesh Shah[2], Indra Napit[2] and Jivan Shakya[1*]

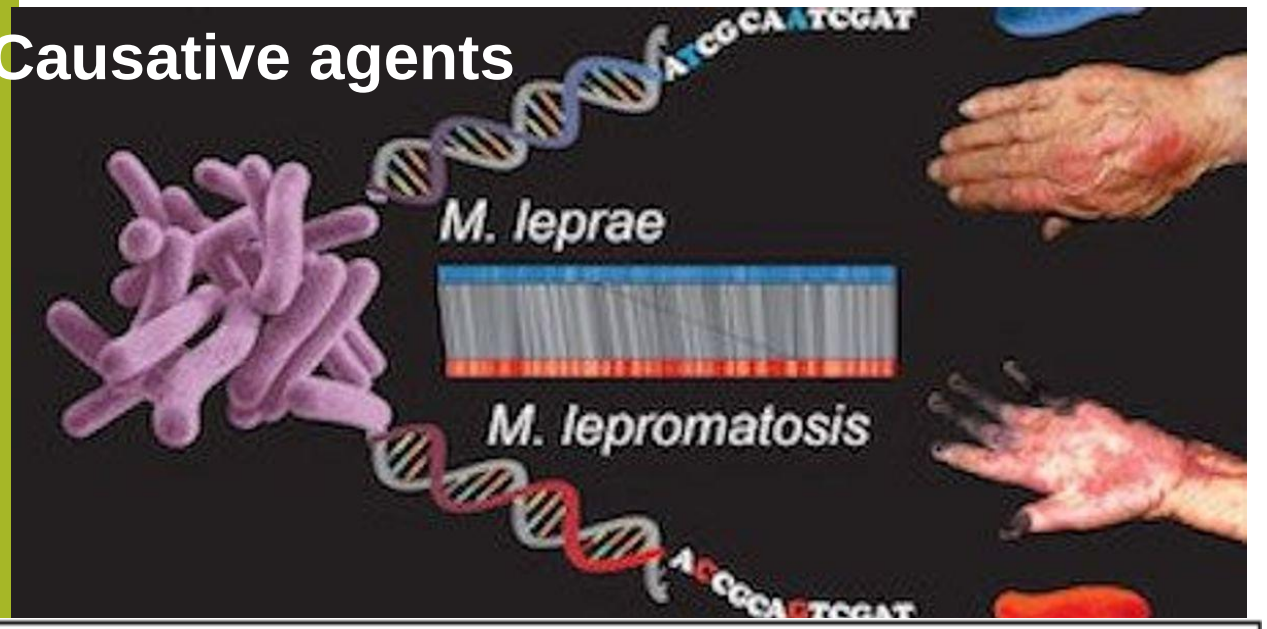
[1]Mycobacterial Research Laboratories, The Leprosy Mission Nepal

[2]Anandaban Hospital, The Leprosy Mission Nepal

[*]Corresponding Authors:- krishusn@tlmnepal.org

jivans@tlmnepal.org

Background – Leprosy as an Infectious Disease



Cardinal Signs of Leprosy

- Hypopigmented or reddish skin patch with definite loss of sensation
- Thickened/enlarged peripheral nerve with loss of sensation +/- muscle weakness *(Nerve thickening without any associated signs and symptoms should not be diagnosed as leprosy)*
- The presence of acid-fast bacilli (AFB) in slit skin smear

Global Concern (WHO, 2025)

- Occurs in more than 120 countries
- Around 200,000 new cases reported every year

Situation in Nepal FY 2079/80 (DoHS, 2024)

- 2523 new leprosy cases
- NCDR 8.5 per 100,000 population,
- Prevalence rate (PR) was 0.8 per 10,000 population

Challege of AMR surveillance in Leprosy

- Unlike other bacteria, M. leprae cannot be cultured in vitro. Hence, in vitro testing of antibiotic sensitivity cannot be done by disc diffusion.

Tools For Monitoring AMR

- **In vivo Method**
  - **Mouse-foot pad (MFP) assay: (phenotypic)**

- **Molecular methods**
  - **Sequencing**
  - **Probe hybridization (HAIN Hybridization Assay)**
  - HRM assay (High resolution melt)
  - Deeplex MycLep Assay
  - Whole genome sequencing

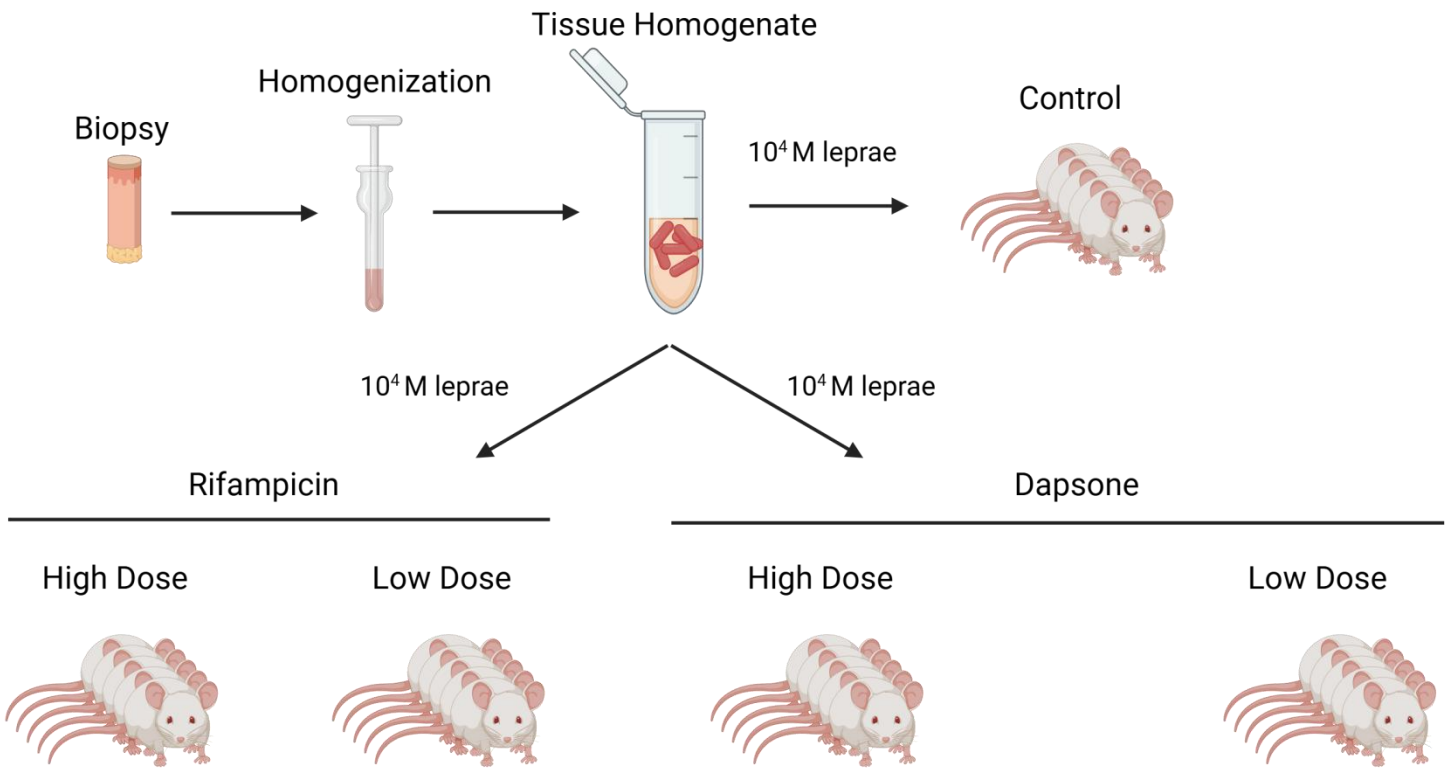
Objectives:

- **General Objective**
  - To report on AMR status of *M. leprae* in recurrent leprosy cases over the period of 2017-2023
- **Specific Objectives:**
  - To report on spatial distribution of leprosy recurrence in Nepal over the period of 2017-2023 using MFP technique
  - To report results of AMR surveillance by MFP, Hain Leprae DR kit and by sequencing.
  - To compare between MFP and molecular techniques of AMR surveillance in leprosy

Methodology

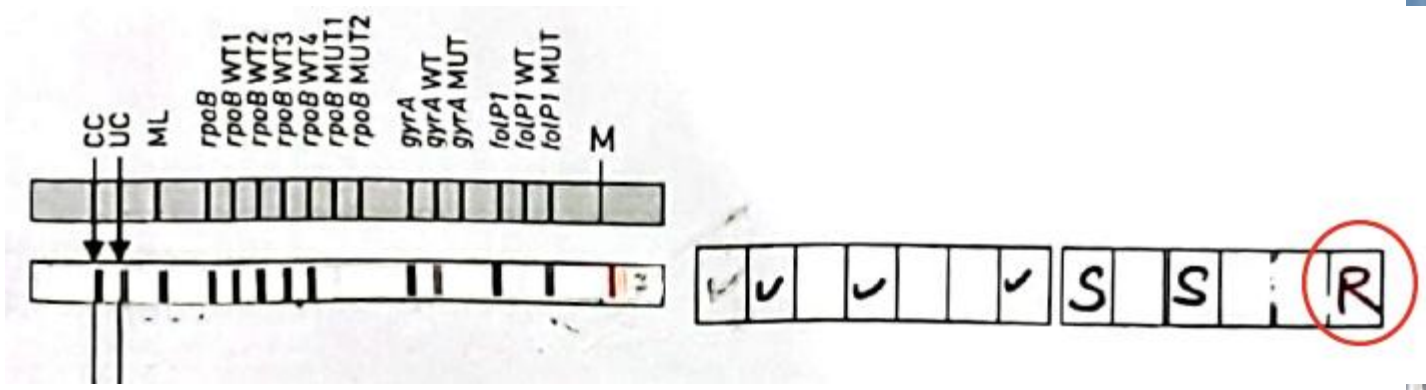
- A retrospective review of the records of Multibacillary (MB) relapse patients from 2017 January to 2023 December was done and analyzed
- Anandaban Hospital has been allocated as a **relapse confirmation center** in Nepal as well as one of the **sentinel site for Leprosy AMR Surveillance**
- For MFP Assay, biopsy sample from relapse leprosy cases were inoculated into Swiss albino mice to assess the viability and drug susceptibility for Dapsone (DDS) and Rifampicin
- Data from Genotype LepraeDR and Sanger sequencing were analyzed to compare with the result of MFP Assay

Methodology for Mouse foot pad Assay



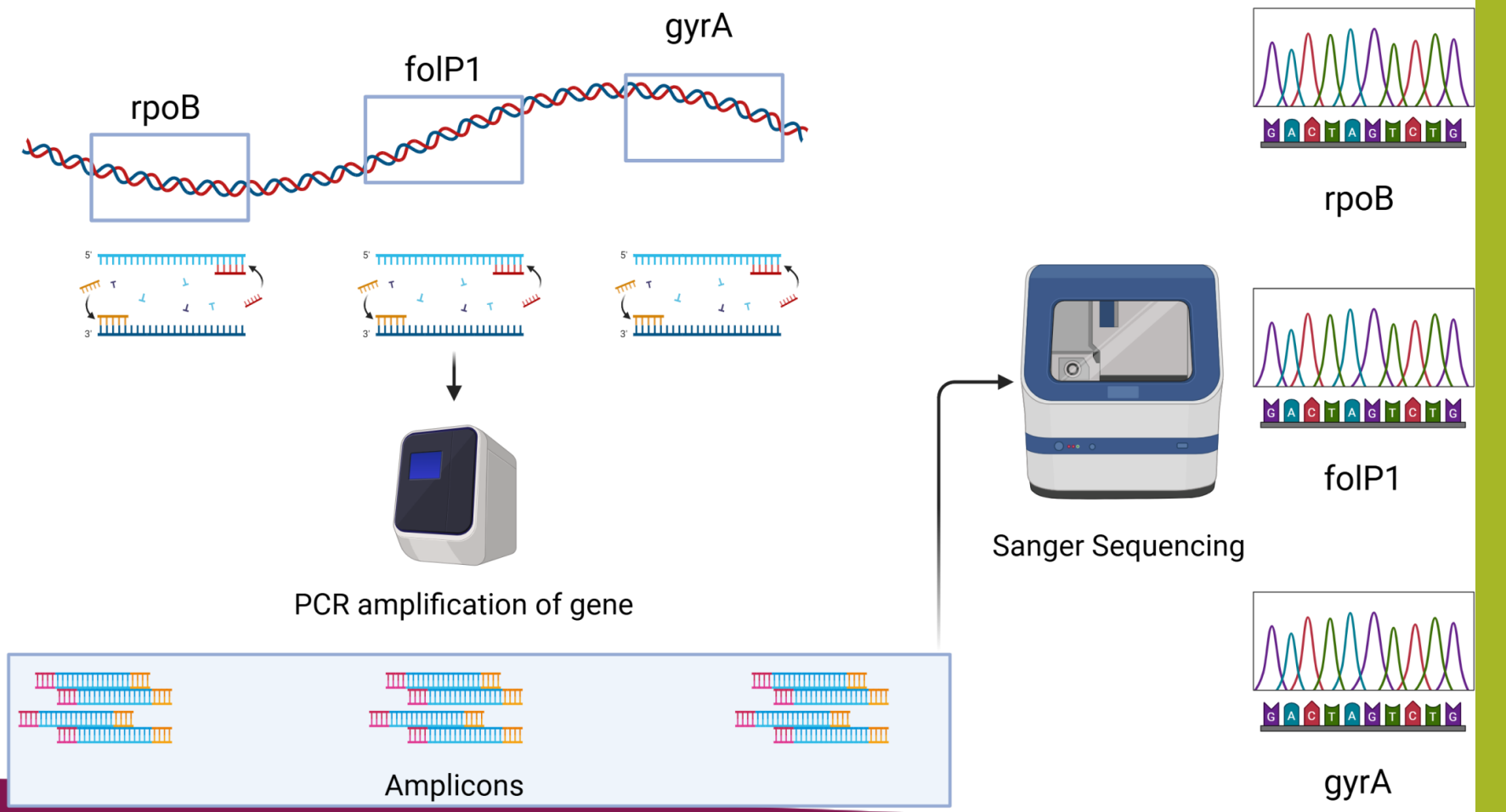
Inoculation into Hind Footpad

Depicting GenoType LepraDR



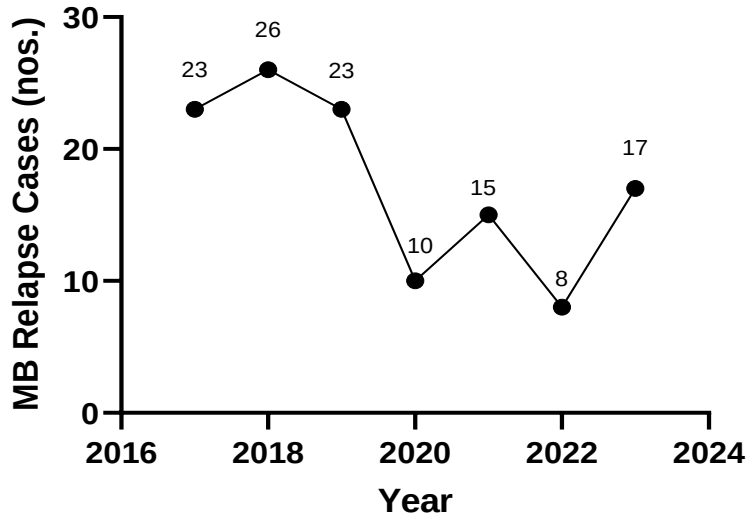
Sequencing

- **Genes:**
  - rpoB (Rifampicin)
  - folP1 (Dapsone)
  - gyrA (Fluoroquinolone)



Results

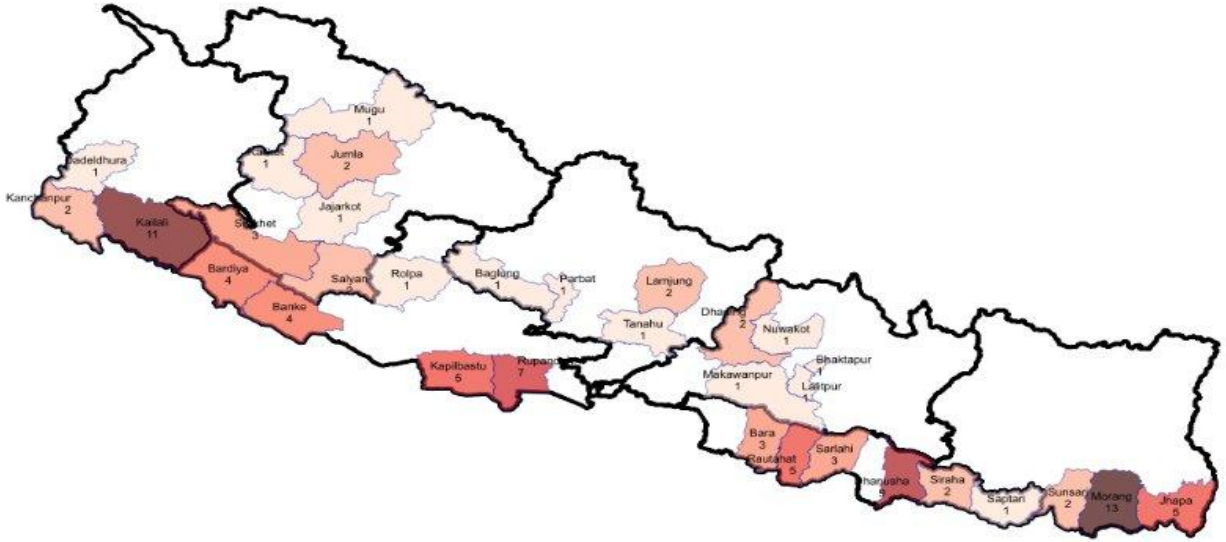
Trend of MB relapse cases beween 2017-2023



- Most cases of MB relapse between 2017 and 2023 were from Morang, Kailali, Dhanusha and Rupandehi

- The last five-year prevalence of Morang, Kailali, Dhanusha and Rupandehi districts is > 1 per 10,000 population (DoHS Annual Report)

Spatial Distribution of MB relapse cases (2017-2023)

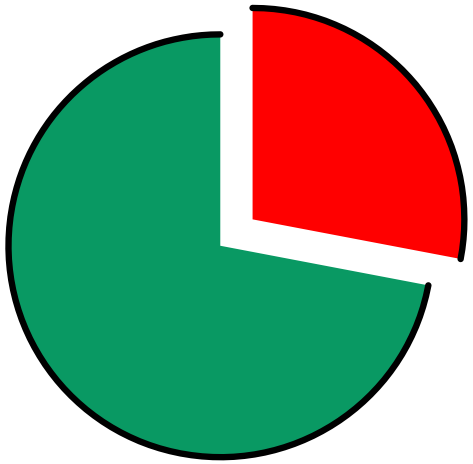


Mapping of Leprosy Prevalence Rate (FY 2079/80)

Figure 10.11 District based Leprosy prevalence rate in FY 2079/80

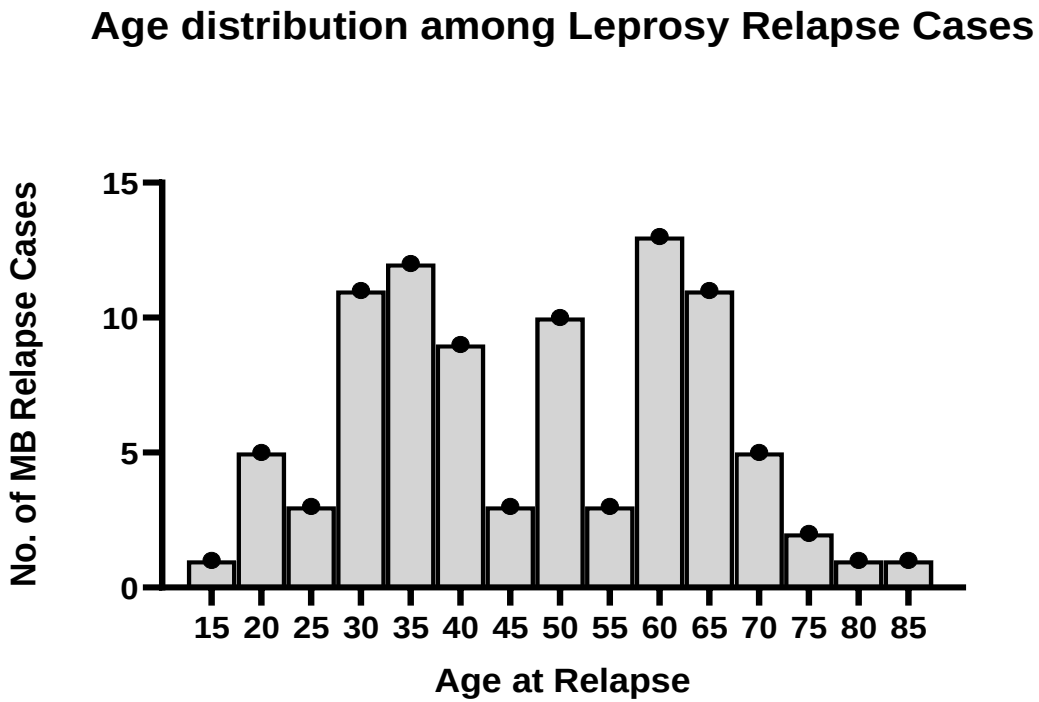
Source: HMIS/DoHS

Results



28.00% Female
72.00% Male

Total=125



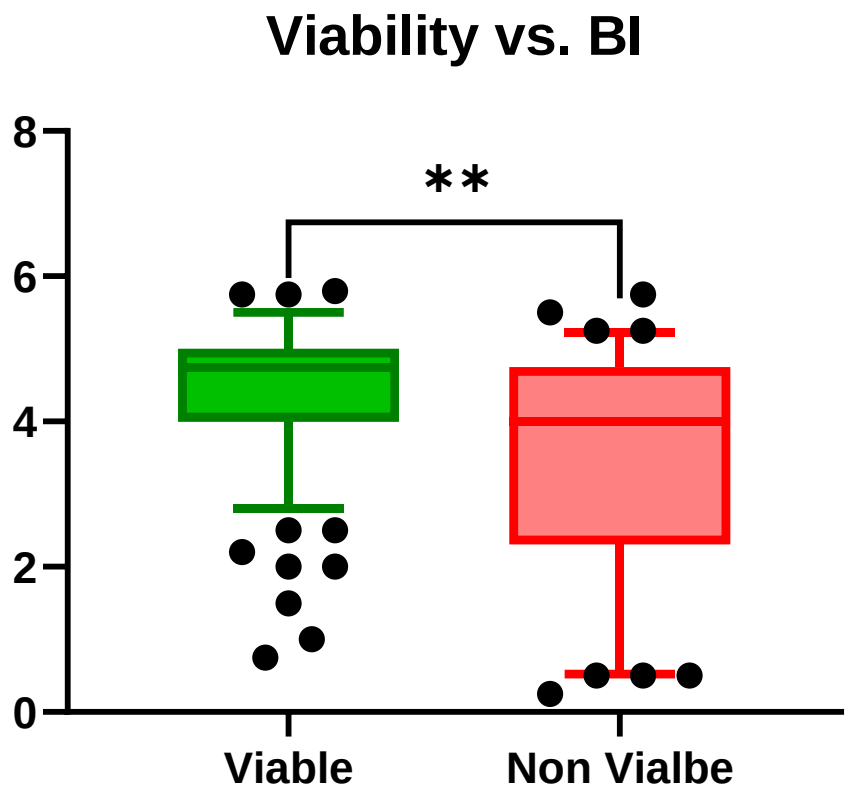
- Recurrent Leprosy were more commonly seen in Males than in Females.

- Recurrence of leprosy occurred more often at ages 35 and 60.

Results

- Median age of relapse in Males was 48.5
- Median age of relapse in Females was 45
- No significant difference was found between males and females for age at which relapse occurred.

Results



- 72 % had a BI ≥4+ at the time of Relapse.
- Viability of M. leprae in Mouse Footpad was significantly determined by the BI at relapse.

Results

Drug Resistance Testing using Mouse Footpad Assay

No. of Experiment showing Viability	Growth in Dapsone		Growth in Rifampicin	
	High dose (0.01%)	Low dose (0.0001%)	High dose (0.1%)	Low Dose (0.05%)
85 (68%)	**0**	**2**	**0**	**1**

Results

Comparison of drug resistance between MFP and molecular methods

Testing	Method	Total Tested	folp1 (Dapsone resistance)		rpoB (Rifampicin resistance)	
			Sensitive	Resistance	Sensitive	Resistance
Genotypic	Sanger Sequencing	47	45	**2**	47	0
	Genotype LepraDR*	17	17	0	17	0
Phenotypic	Mouse Foot Pad (MFP) Assay*	85**	85	0	85	0

*Available at Mycobacterial Research Laboratories, Anandaban Hospital
**Experiment that showed viability

Take away Message

- Leprosy Recurrence is one big challenge to achieve leprosy elimination.
- Mouse Foot Pad Assay although time consuming remains a critical phenotypic tool for AMR surveillance in unculturable bacteria like *M. leprae*
- Combining phenotypic and genotypic methods like Sequencing and/or Genotype LepraeDR strengthens early resistance diagnosis eventually contributing to better leprosy case management.
- Strengthening AMR surveillance for both New and Retreatment cases is crucial for achieving Zero Transmission and Zero Disability.

Acknowledgement

- Leprosy Affected People
- Dr. Yuji Miyamoto, Dr Matsuoka Masanori, Dr. Manabu Ato (Leprosy Research Center, National Insititute of Infectious Diseases, Japan)
- The Leprosy Mission Great Britain, UK

Thank you !

- Questions!

I have been working as a Research Officer at Mycobacterial Research Laboratories (MRL), The Leprosy Mission Nepal for last 2.5 years. I received a Master of Science with a specialization in Medical Microbiology from Tribhuvan University in 2016. I have a keen interest in infectious disease and antimicrobial resistance with focus on Leprosy and Drug resistance Surveillance