

Laboratory aspects of drug resistance in Leprosy

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Historical background and Issues

- The determination of drug resistance in leprosy has been complicated due to its non-cultivability of *M.leprae* in any accepted *in-vitro* medium system.
- Since 1960, Mouse foot pad has been the acceptable tool to detect and determine the levels of resistance to various anti-leprosy drugs.
- The scenario has undergone many changes during the last 25 years. In 1970s and early 1980s, there was increase in drug resistance to sulphones and also some cases were reported to be resistant to other drugs.

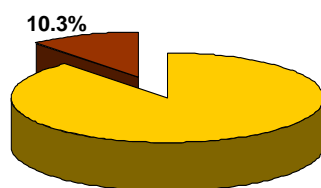
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Changing profile of disease

- WHO led multi drug treatment (MDT) campaign all over the world has produced major change in the situation.
- Overall the prevalence of the disease has decreased.
- **The proportion of the bacillated cases has decreased.**
- This leads to a lesser usefulness of mouse foot pad to monitor drug resistance even as a surveillance tool.

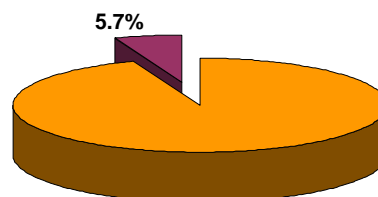
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2003-2005 survey



■ smear negative ■ smear positive

2006-2007 re-survey



■ smear negative ■ smear positive

- In the same population in the last survey 1, 80,982 persons were examined. In the present survey 2,05,511 persons have been examined. However this is about 70% of the census population.
- Among the 435 cases detected 10 patients had reactions (9 RR and 1ENL) before and during treatment. In the same population In the last survey 46 patients had reaction during treatment (42 RR and 4 ENL).

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Alternatives

- Considering this situation, one has to debate and decide about the utility of other techniques which could be used to undertake the drug susceptibility testing.
- Over the years, **several phenotypic and genotypic markers** have been described for determination of **viability and drug susceptibility testing**.

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Alternative methods for determination of viability and DST

- These include growing the *M.leprae* in **macrophages and/or in media system showing limited growth** and use various markers like enzymes, ATP, isotope uptakes, change in the receptors etc to detect viable bacillus.
- All these methods require certain minimum number of bacilli and again **will have a limited usefulness in the current scenario** when multibacillary cases have become lesser in number.
- **MFP feasible in smear negative** cases but the **number of animals** required for getting **initial growth, amplification** and **DST** will be large/ not established as a reproducible procedure.

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Appropriate methods in current scenario

- In the present situation with paucibacillary disease constituting major proportion (> 90%), molecular methods for direct detection of mutations in the genes responsible for resistance like *rpoB* for rifampicin, *foI/P* for dapsons, *gyrA* for quinolones will have a greater scope of application.

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Sulphone resistance in patients attending JALMA OPD (Pre and Post 1990 by Mouse foot pad

Pre 1990			1995-2000		
No. of patients	Med Res	High Res	No. of patients	Med Res	High Res
84	7	16	77	1	3
Percentage	(8.3%)	(19.1%)		(1.3%)	(3.9%)
	23 (27.4%)			4 (5.19%)	

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Country	Author	Methods used	Samples	Resistance (%)		
				Sulphone resistance	Rifampicin resistance	Clofazimine
Nepal	Butlin et al 1996	MFP	157	6.8 (PR) 47.1(SR)	nil	
South India	Ebenzer et al 2002	MFP	265 (1987-97)	4.88 30(PR) 48(SR)	1.36 17.4(PR) 4.35(SR)	2.44 30(PR) 13(SR)
France	Cambau et al 2005	MFP	38	42 6 /6 strains HR 3/4 IR 1/6 LR	ND	ND
Nepal	Butlin et al 1996	MFP	157	6.8 (PR) 47.1(SR)	nil	ND
South India	Ebenzer et al 2002	MFP	265 (1987-97) JALMA (ICMR)	4.88 30(PR) 48(SR)	1.36 17.4(PR) 4.35(SR)	ND

Molecular methods for detection of drug resistance in leprosy

PCR SSCP (Honore et al 1993)

PCR and sequencing (Ramasoota et al 2000, Zhang et al 2005)

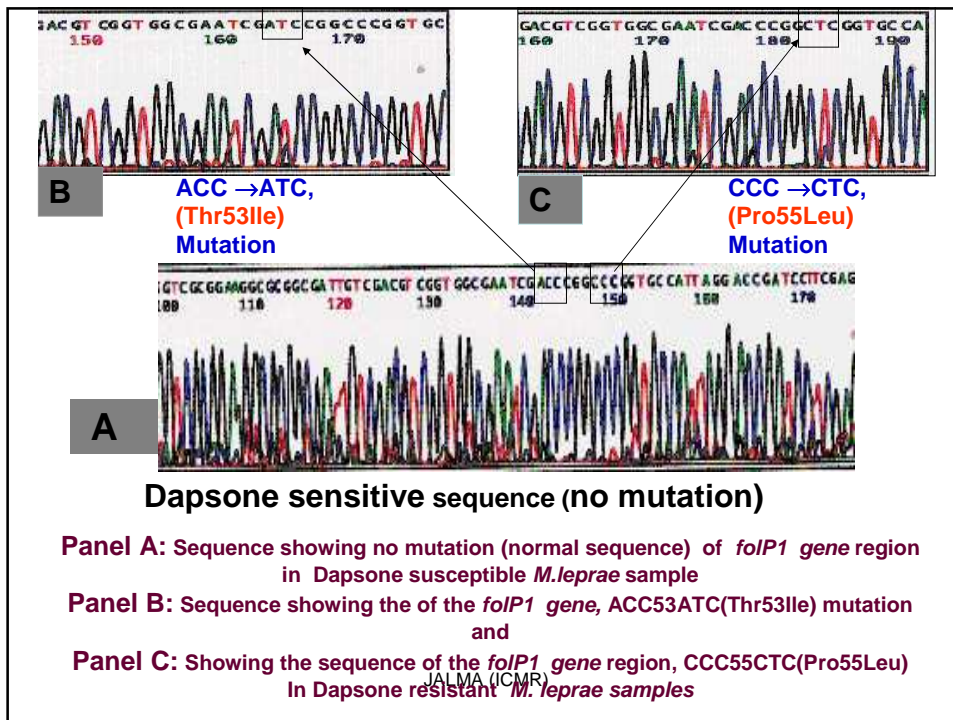
DNA Heteroduplex analysis (Williams et al 2001)

Touch down PCR (Kim et al 2003, You et al 2005)

Reverse line probe assay (Sapkota et al 2006)

DNA Microarray (Suzuki and Matsuoka 2006)

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Mutations in *rpoB* & *folP1* in *M. leprae* in JALMA/ BLP/ SLRC/ Kolkatta study

Resigns	(Sample No)	PB		MB		Mutation Detection						
		+ive	-ive	+ive	-ive	<i>rpoB</i> Gene			<i>folP1</i> Gene			
						Amplified (38)		NA (10)	Amplified (37)			NA (11)
						NM	NRS		Mutation	NM	NRS	
Mumbai	11	2	-	9	-	6	1	4	ACC→ATC ;Thr53Ile (1)	5	1	4
Kolkatta	10	1	1	8	-	6		4		5	1	4
Karigari	11		1	9	1	10		1	CCC→CTC ;Pro55Leu (1)	6	3	1
JALMA	16	3	5	6	2	13	2	1		9	5	2
Total	48											

NA=Not Amplified; NRS= Not Readable Sequence
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Country	Author	Methods used	Samples	Resistance (%)				
				fol P	rpoB	folP+rpoB	fol P+gyr A	23S rRNA
Korea	You et al 2005	Touch down PCR, SSCP	104	19.2	1	2.89	1.92	nil
Pakistan	Kai et al 2004	PCR seq		4/18 22	1/10 10	ND	ND	ND
Thailand	Rama soota et al 2000	PCR Seq	9	ND	Multiple mutations	ND	ND	ND
Japan, Haiti, Indonesia, Pakistan, Philippines	Maeda et al 2001	PCR Seq	88	21.59	29.54	13.63	3.4	2.22

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