Reliability of Anti-TB Drug Susceptibility Testing

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Reliability of DST

- Poor Predictive Values of DST Results (not well correlate with treatment outcomes)
- Poor Test Reliability

Reliability of DST

- Phenotypical Resistance
  identify by
  - conventional growth based method
  - rapid growth based method
  - molecular technique
- Clinical drug resistance
  - by calibration with PS and PR strains

In vitro Test Reliability

-technical accuracy/ reproducibility

1. In incorporating the desired (critical) concentration of potent drug into medium
   - stability variation, antibacterial activity variation in different media
2. In standardizing the size and viability of inoculum
3. In standardizing the test system
4. In incubation, reading, interpreting, and reporting

* Internal/ external QA

External Quality Assessment of *DST* in Global *DRS* Network

- **Proficiency testing with a panel of duplicate set of *MTB* strains**

(1)Detection of resistance (sensitivity)
(2)Detection of susceptibility (specificity)
(3)Over-all concordance
(4)Reproducibility (duplicate set of 10 strains + 10 non-duplicate strains= 30)

- **Cross-checking of *MTB* strains tested at labs being controlled: discrepant results may settle at the 3rd reference laboratory**

The 14th Round Proficiency Test Results of 17 Laboratories in SNRL Network

(*data (2007) from Armand Van Deun*)

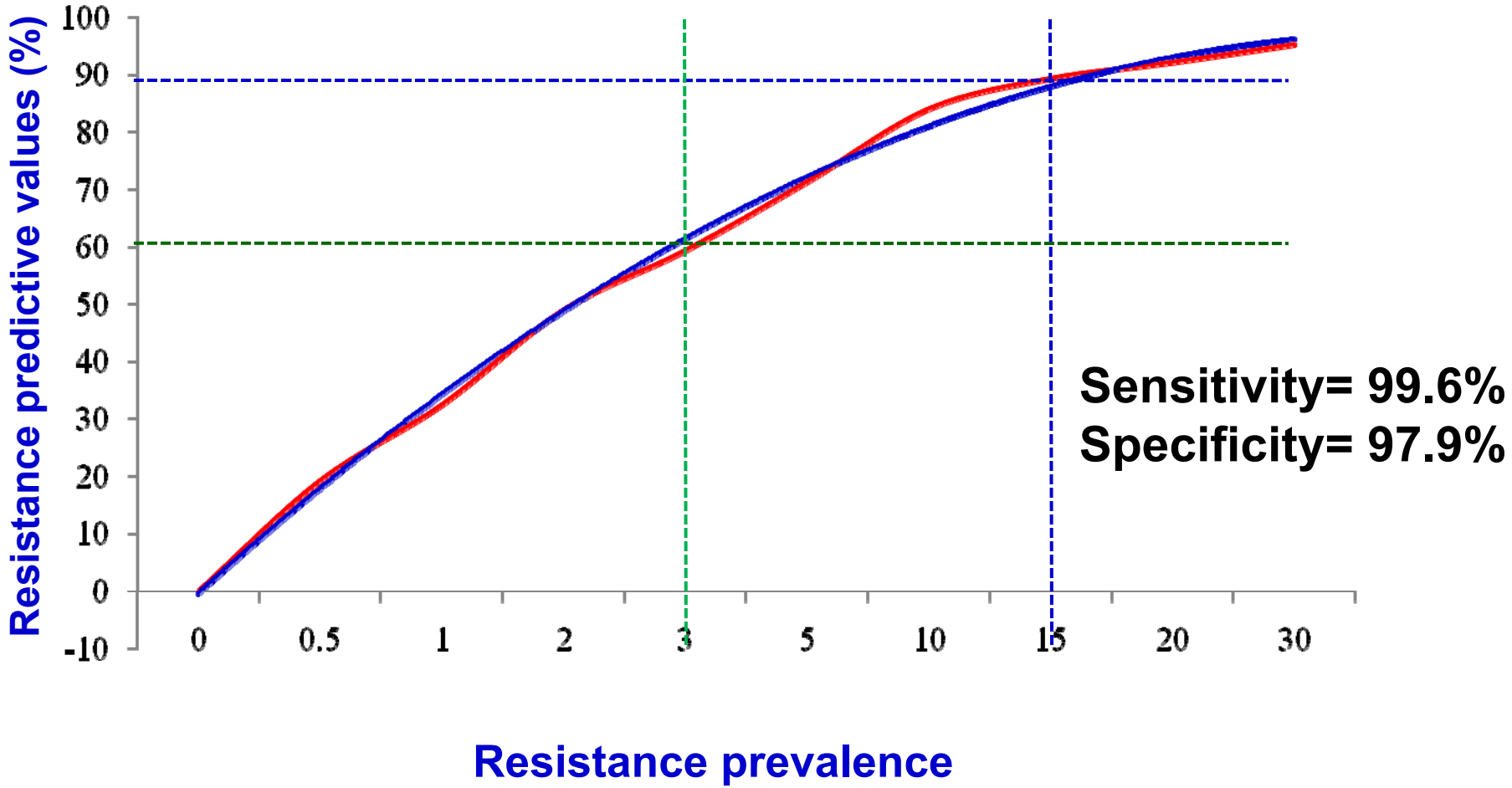
		Sensitivity	Specificity	Efficiency	Reproducibility
Isoniazid S=13 R=17	Average (%)	99	99	99	98
	100%	13	14	11	14
	<95%	4	3	1	3
Rifampicin S=10 R=17	Average (%)	99	99	99	98
	100%	14	14	12	14
	<95%	3	3	2	3
Streptomycin S=11 R=16	Average (%)	97	91	95	94
	100%	12	11	7	9
	<95%	5	6	8	8
Ethambutol S=17 R=4	Average (%)	88	97	96	97
	100%	11	13	8	11
	<95%	6	4	7	6

Numbers in blue indicate the number of laboratories

Resistance Predictive Values of Rifampicin Susceptibility Tests

based on 14th Round PT data (2007) of SNRL network

(*data from Armand Van Deun*)



Agreement of DST Results to Isoniazid and Rifampicin of the 51 National (or Regional) Reference Laboratories

(WHO 4th Report of Global Drug Resistance, 2008)

% Agreement	Isoniazid	Rifampicin
100	38 (77.6)	37 (72.5)
95-99	6 (12.2)	9 (17.6)
90-94	4 (8.2)	5 (9.8)
<90	1 (2.0)	0
Total number of labs	49 (100.0)	51 (100.0)

Resistant Predictive Values of Second-line Anti-TB Drugs

Test drug	Sensitivity (%)	Specificity (%)	Predictive Values (%) of Resistant Results at the Prevalence rates:			
			1%[1]	5%[1]	10%[1]	30%[1]
KM[2]	100	90	9.2	34.5	52.6	81.1
CPM[2]	100	96	20.0	56.8	73.5	91.5
OFX[2]	100	97	25.0	64.1	78.7	93.5
ETH[2]	79	91	8.2	32.0	49.4	79.0

[1] Prevalence rates

[2] Results of SLDST proficiency tests done once in 2004 (coordinated by Dr. Nuria Martine Casabona) (participated SRL=9)

[3] Based on sensitivity/ specificity of 9 rounds proficiency tests of SRLs, 1994-2002

Predictive values of SLDST results

Krüüner A, et al. JCM 44:811, 2006

Drug	Sensit-ivity	Speci-ficity	Predictive values of test results Resistant	Susceptible
Amikacin (1.0)	96	99	96	99
Capreomycin (1.25)	92	98	92	98
Ofloxacin (1.0)	100	100	100	100
Rifabutin (0.5)	98	100	100	75
Prothion- (2.5)	75	98	82	98
amide (5.0)	63	99	83	98

Numbers indicate per cents. No of strains studied=133

Proficiency Test Results of the 2nd-line DST of 5 SNRL

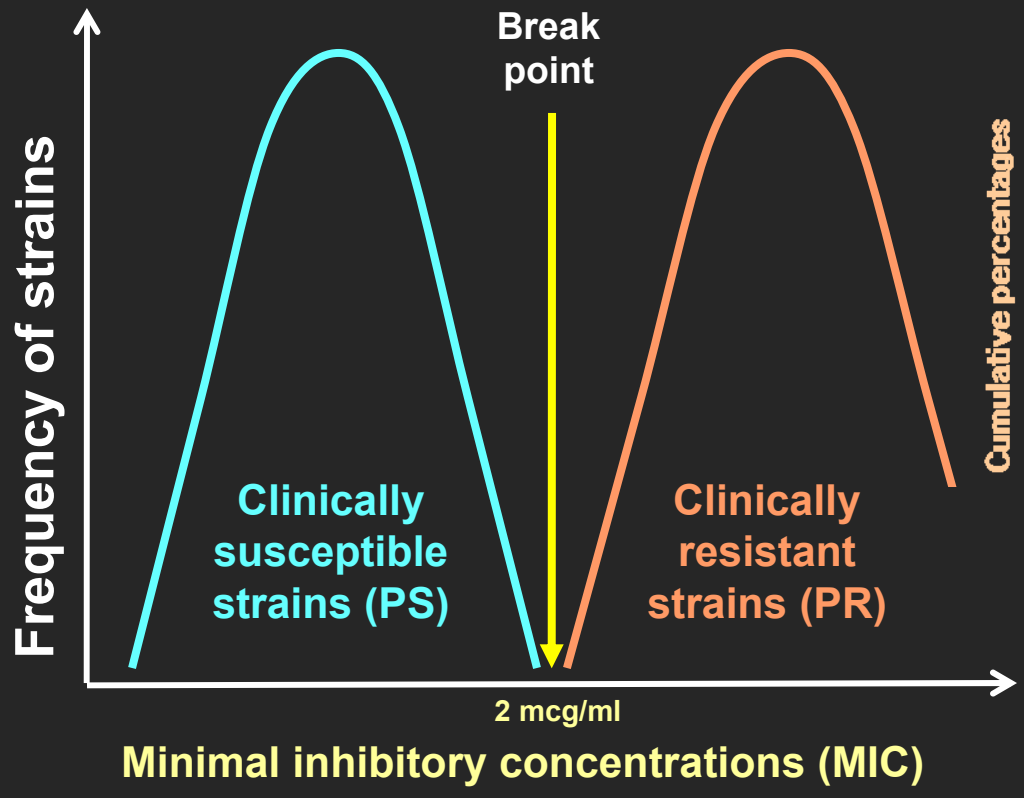
(*data from Armand Van Deun*)

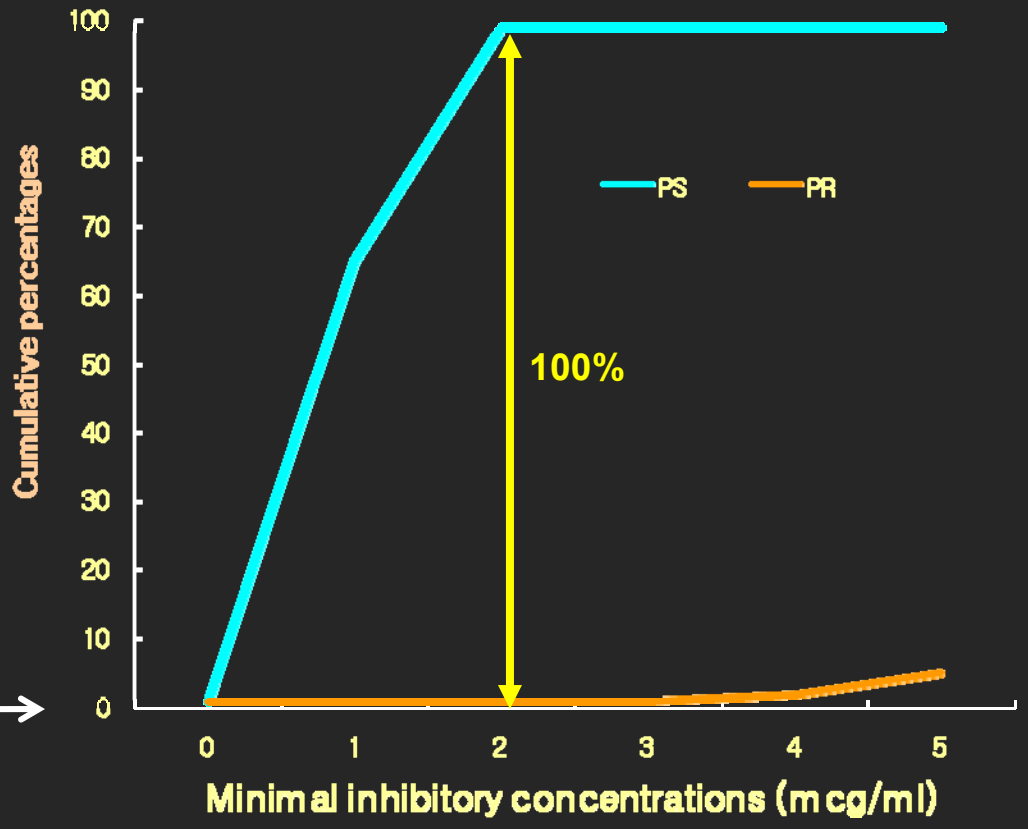
	Agreement (%)	Kanamycin (S=9; R=11)	Amikacin (S=14; R=6)	Capreomycin (S=10; R=10)	Ofloxacin (S=17; R=3)
Seansitivity	100	4	3	3	4
	95-99	0	0	0	0
	90-94	1	0	0	0
	<90	0	1	1	1
Specificity	100	5	4	4	4
	95-99	0	0	0	1
	90-94	0	0	0	0
	<90	0	0	0	0
Efficiency	100	4	3	3	3
	95-99	1	1	0	2
	90-94	0	0	0	0
	<90	0	0	1	0
Reproduci-bility	100	5	3	4	4
	95-99	0	0	0	0
	90-94	0	1	0	0
	<90	0	0	0	0

Criteria of Resistance in Drug Susceptibility Testing

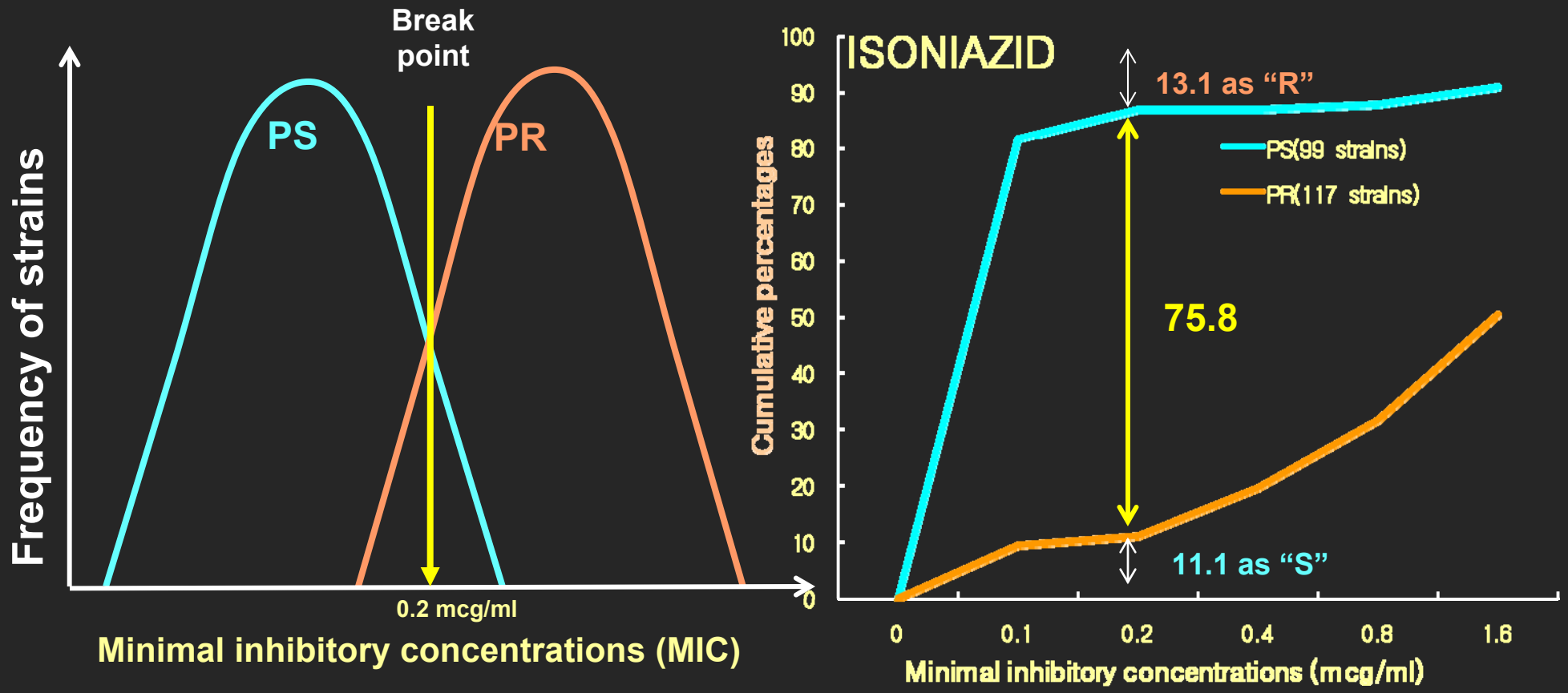
***Calibrate in different test systems with representative samples of probably resistant (PR) and probably susceptible (PS) clinical isolates of* M. tuberculosis**

Distribution of probably susceptible/ probably resistant strains to the various concentrations of drug



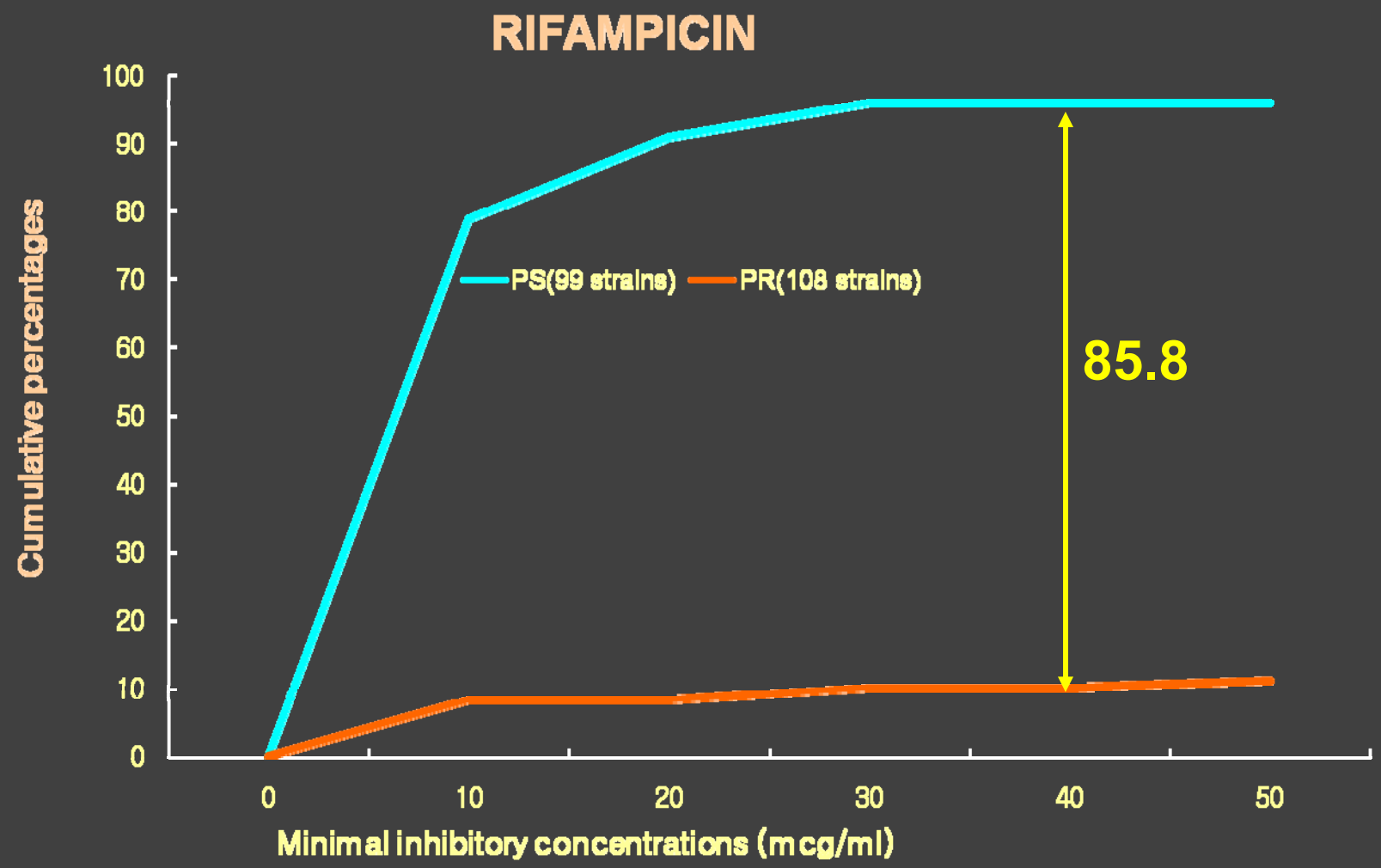


Distribution of probably susceptible/ probably resistant strains at the various concentrations of drug



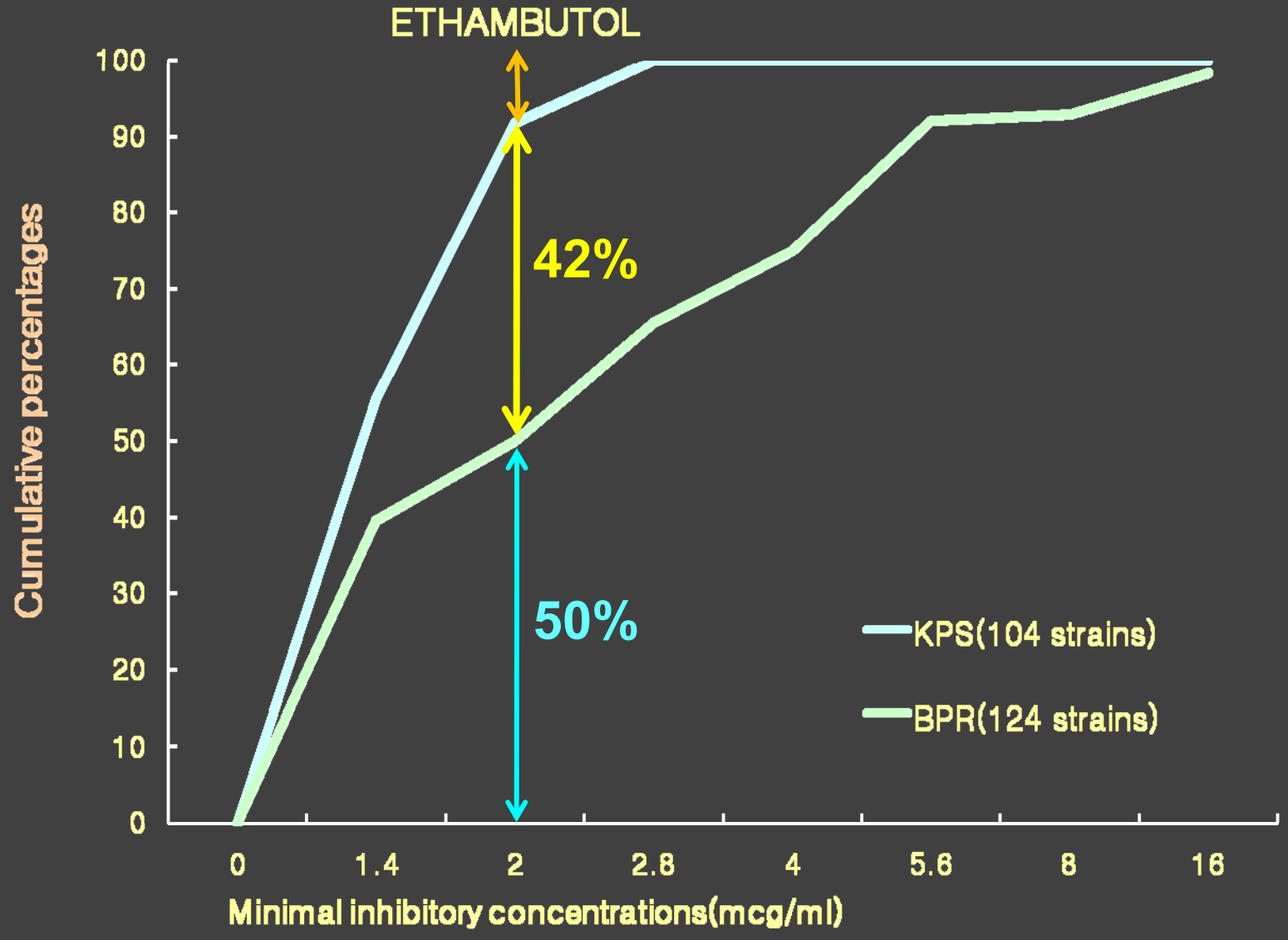
Cumulative Distribution of MIC of PS/PR Strains of *M. tuberculosis* to RFP

(Tests done in L-J medium using the absolute concentration method)

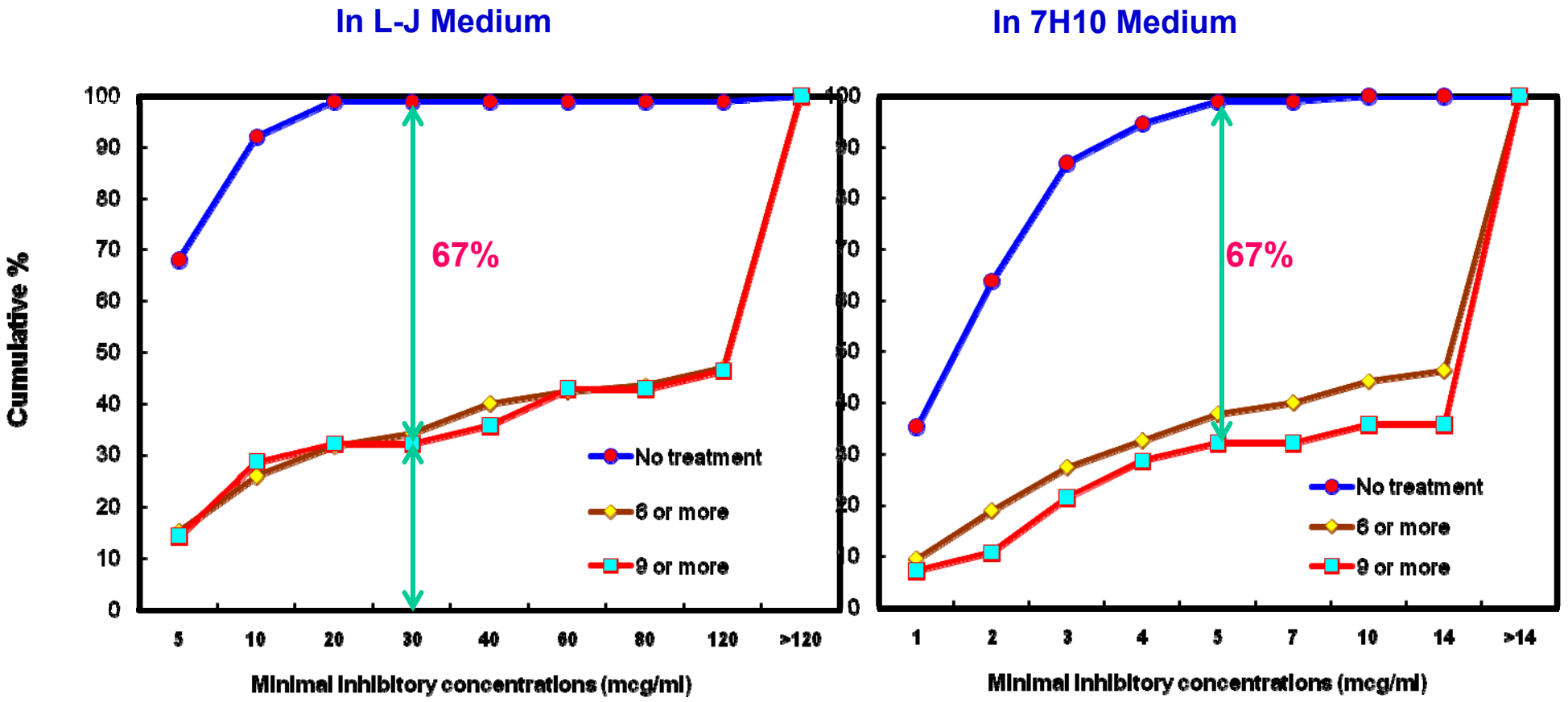


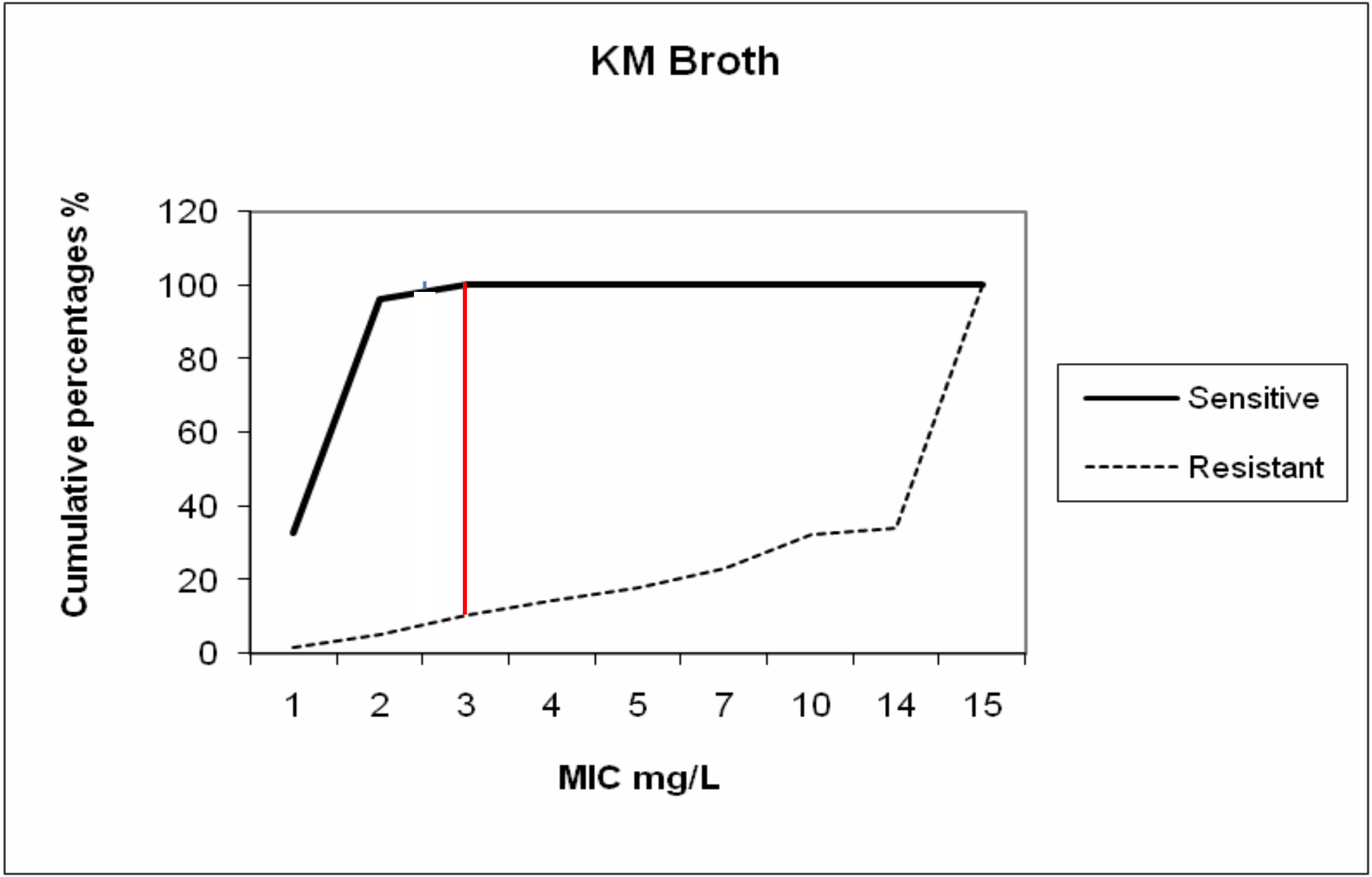
Calibration of Ethambutol Critical Concentrations

(Tests done in L-J medium using the absolute concentration method)

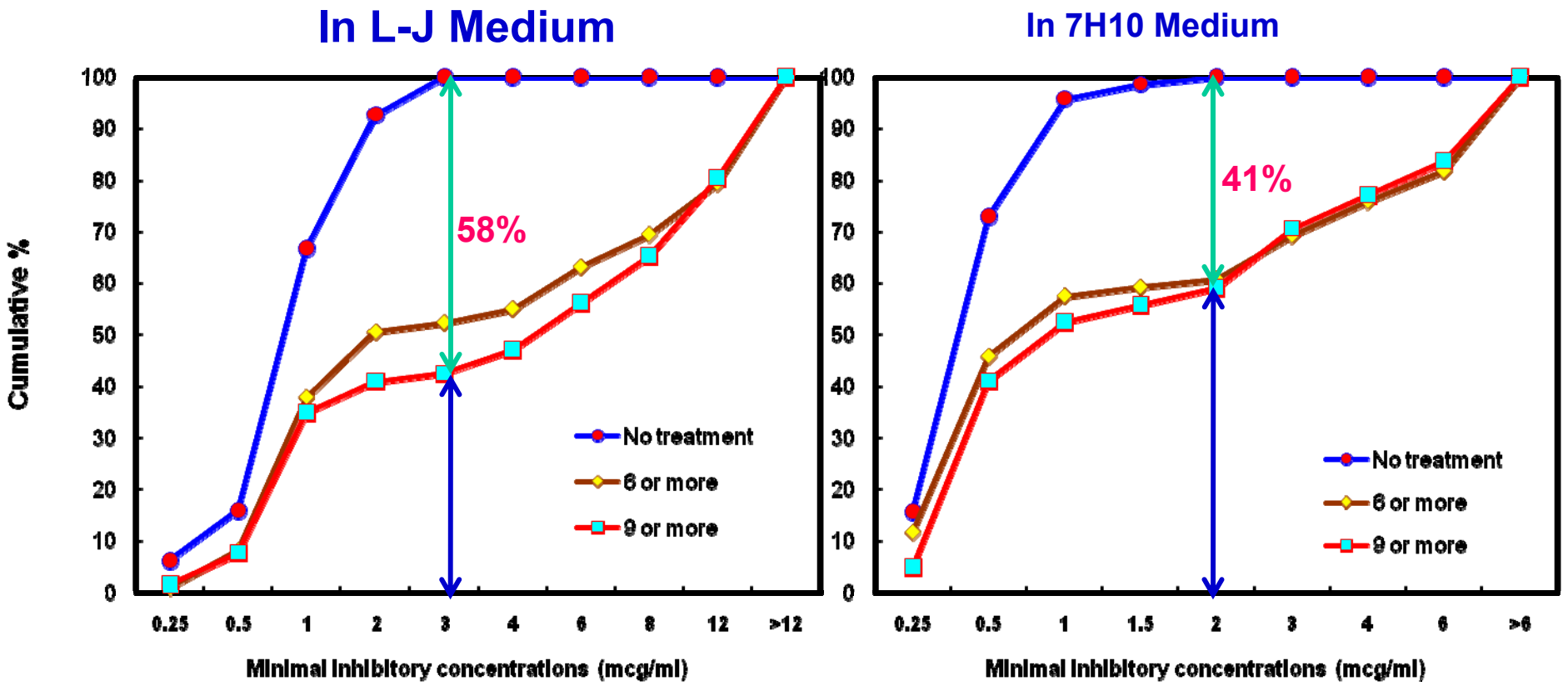


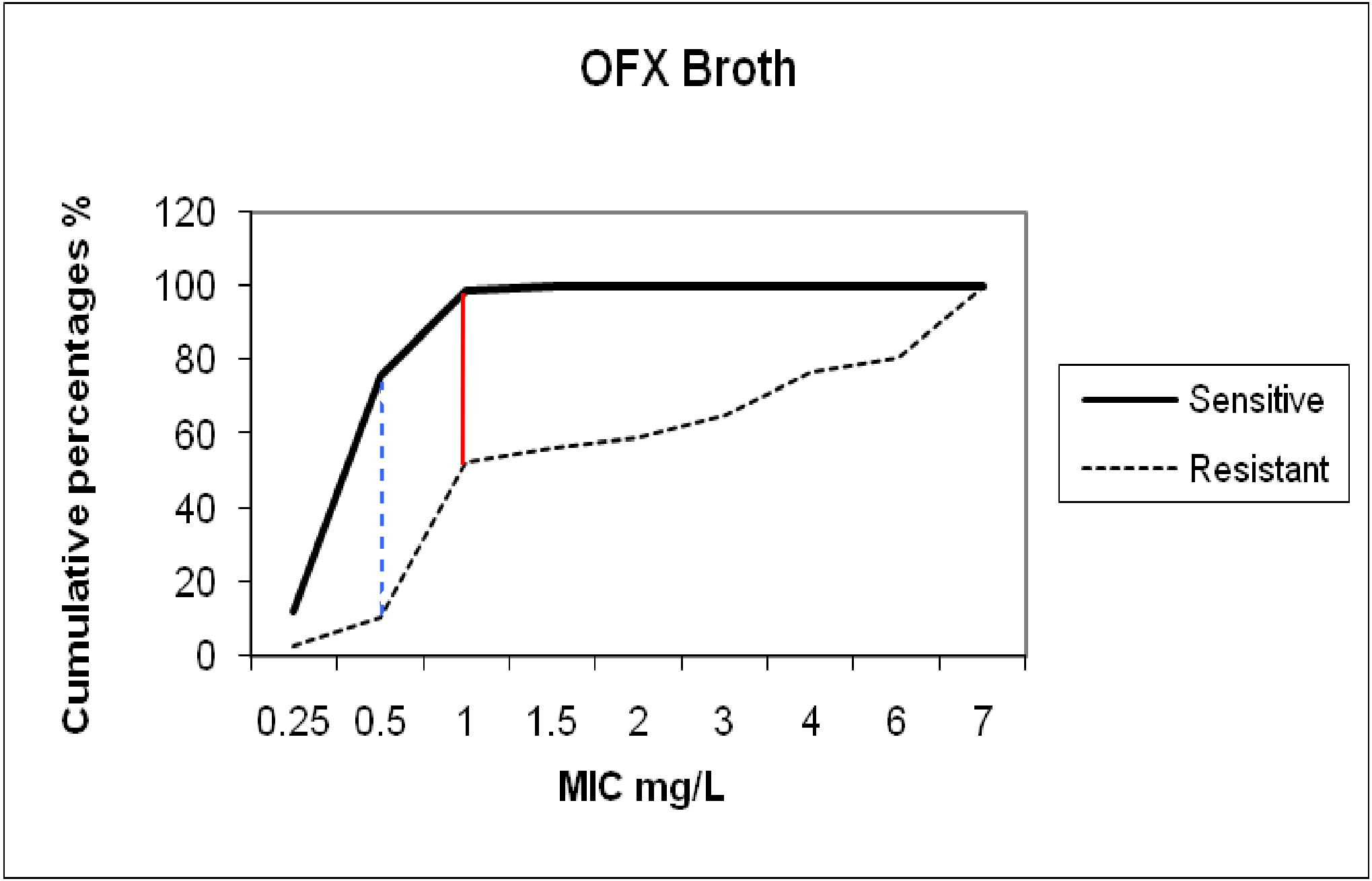
Kanamycin Susceptibility Patterns of Probably Susceptible and Probably Resistant Clinical Isolates of *M. tuberculosis*




KM Broth
Cumulative percentages %
120
100
80
60
40
20
0
1
2
3
4
5
7
10
14
15
MIC mg/L
Sensitive
Resistant

Ofloxacin Susceptibility Patterns of Probably Susceptible and Probably Resistant Clinical Isolates of *M. tuberculosis*



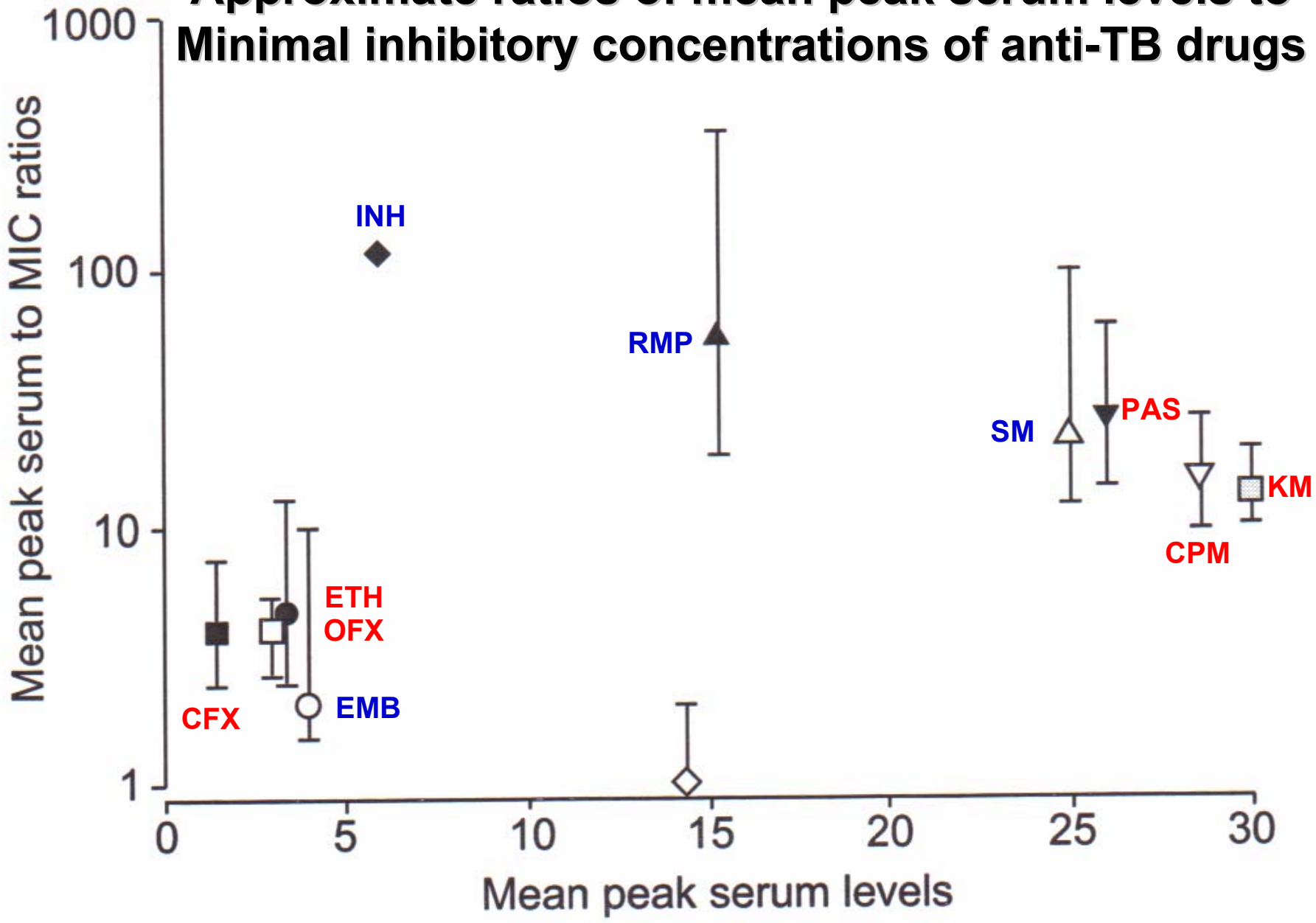

OFX Broth
Cumulative percentages %
120
100
80
60
40
20
0
0.25
0.5
1
1.5
2
3
4
6
7
MIC mg/L
Sensitive
Resistant

Susceptible Results of Clinically Resistant Isolates at Critical Concentrations of 2nd-line Anti-TB drugs

Drugs	In Löwenstain-Jensen medium		In 7H10 agar medium	
	FS(%)	CC (µg/Mℓ)	FS(%)	CC (µg/Mℓ)
Capreomycin	27.6	40.0	31.3	8.0
Cycloserine	81.1	30.0	-	-
Ethionamide	26.5	40.0	23.4	3.0
Kanamycin	31.8	30.0	37.9	5.0
Ofloxacin	52.3	3.0	60.8	2.0
Para-amino-salicylic acid	49.5	1.0	53.8	2.0

CC= critical concentrations; FS= sensitive DST results of PR strains

Approximate ratios of mean peak serum levels to Minimal inhibitory concentrations of anti-TB drugs



Summary and Conclusions

1. Technical reliability (reproducibility)/ clinical relevance of RMP/INH susceptibility tests are high and robust when compare with all other anti-TB drugs
2. Technical reliabilities of KM/ OFX are good, but a considerable number of PR isolates remain susceptible at their CC, requiring a careful use of susceptible test results if a patient has a treatment history of the corresponding drug
3. Attainable peak blood levels of many of anti-TB drugs except INH/RMP are close to MIC of PS, making so fragile to a minute technical variation that their test reliability is poor in general.

Summary and conclusions

4. Careful selection and standardization of test system and methods are essential to improve reliability

5. Strict/ efficient internal/ external QA are also essential to sustain acceptable level of the technical reliability

6. Molecular techniques should be explored to replace or complement the poor reliability of DST through the rigorous search on drug resistance related genes and their mutation sites and development of easy, cheap, and robust test systems to detect those mutations