DST proficiency testing rounds in the Supranational Reference Laboratory Network

Armand Van Deun
The Union &
Antwerp coordinating SRL

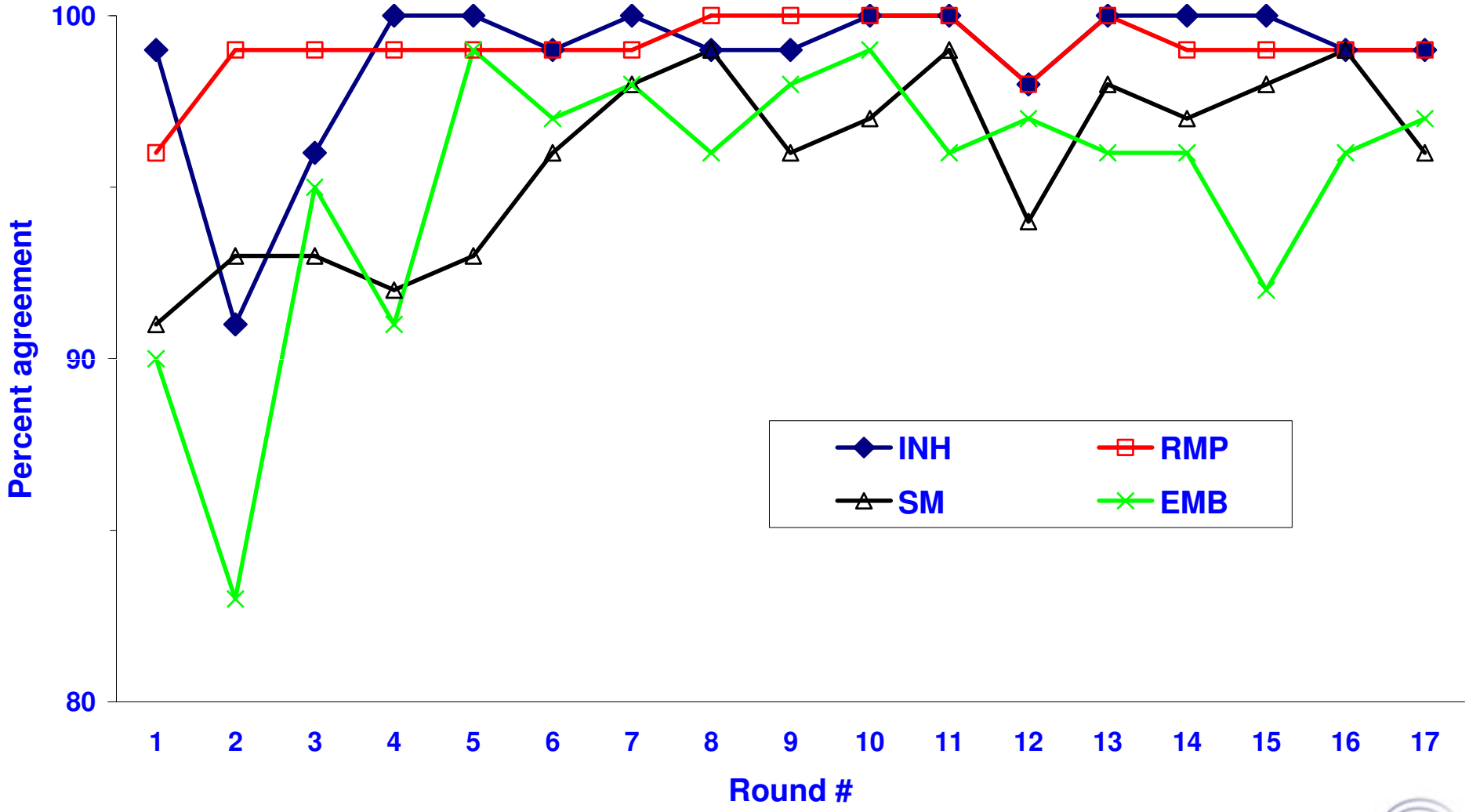
SRL network

- Grown to 32 laboratories (18th round, 2011)
  - 4 new SRL recently
  - 2 in Africa (Kampala, Cotonou); 1 in Asia (Karachi); 1 in Europe (Copenhagen)
- FAQ: how do we become a SRL?
  - confusion regarding role of SRL
  - ToR: help other countries
- DST PT conduct: Antwerp SRL since 1999

Rounds organization

- Annually 30 strains (10 in duplicate)
  - INH, RMP, SM, EMB
- Main second-line drugs added since Rd 14
  - Km, Ak, Cm and Ofx; but no XDR
  - same strains as for first-line
- SRL use their preferred standard method
  - recent rounds: combination of methods encouraged for difficult strains

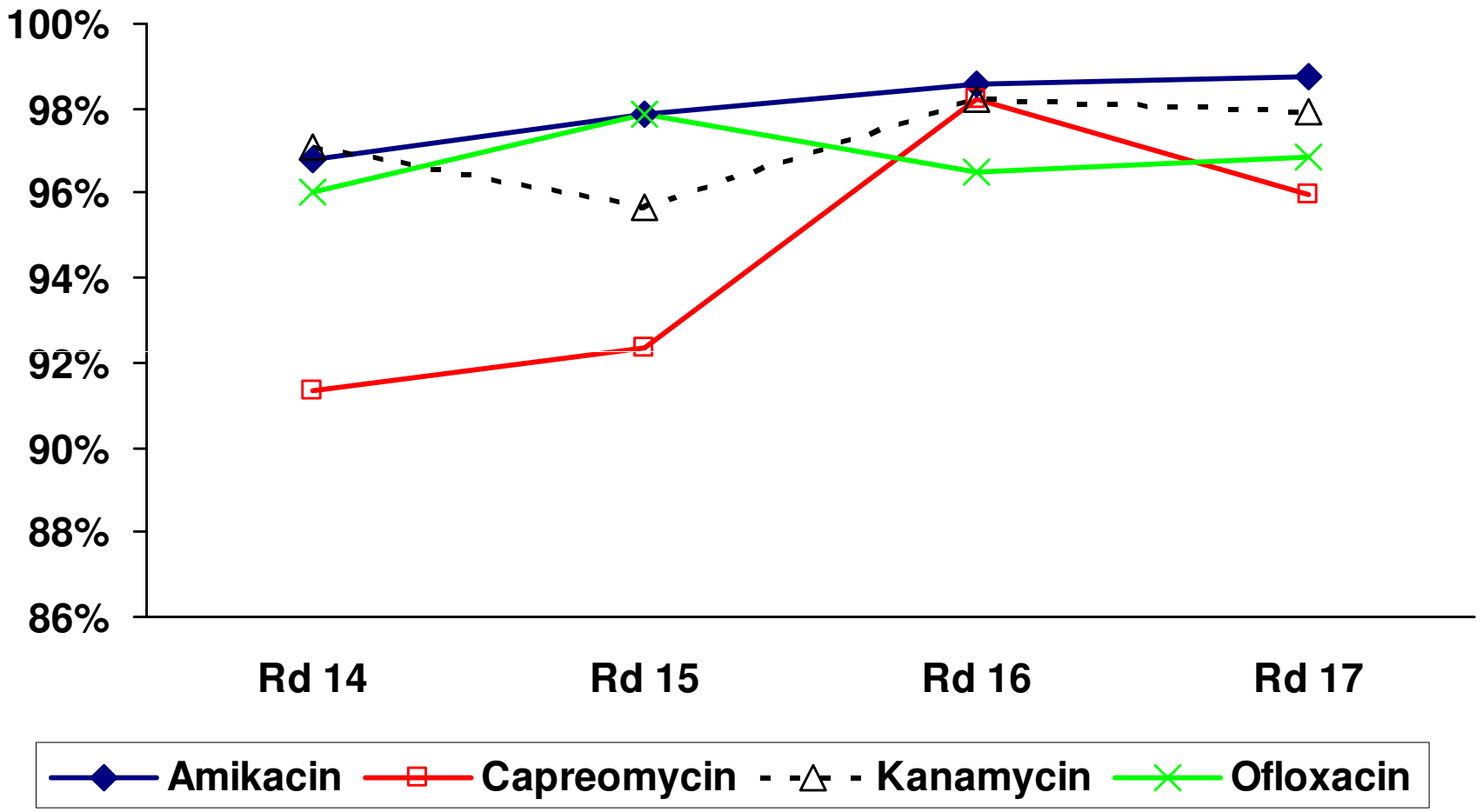
SRLN agreement on first-line DST, round 1 - 17
(<80% concordant strains excluded)



SLD resistant strains, rounds 14 to 17

Round	Km	Ak	Cm	Ofx	Any drug
14	12	6	11	4	33
15	10	7	8	4	29
16	9	8	8	8	33
17	10	6	8	7	31
All rounds	41	27	35	23	126
Total strains	120	120	120	120	480
% resistant	**34%**	**23%**	**29%**	**19%**	**26%**

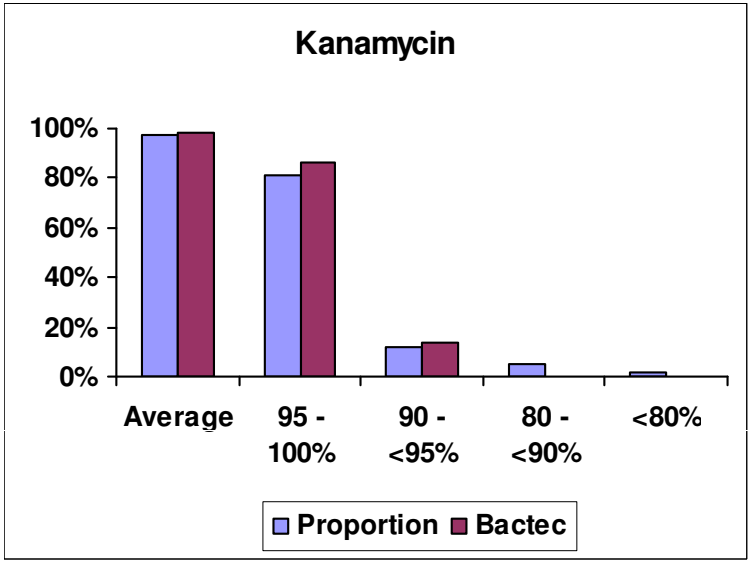
Average accuracy score, trend Rd 14 to 17 of SRLN second-line DST PT

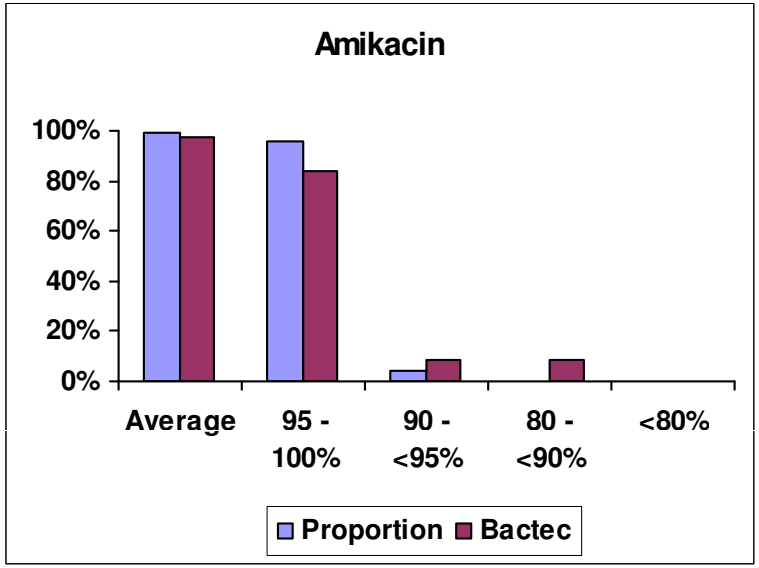


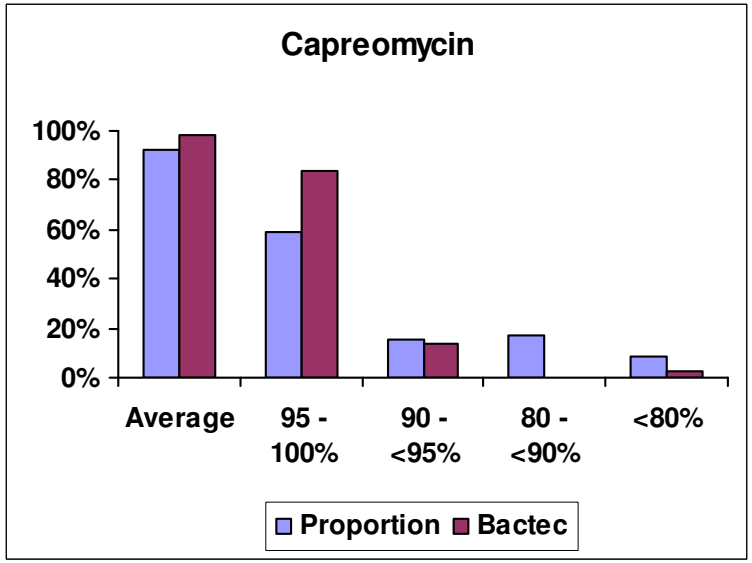
Methods used

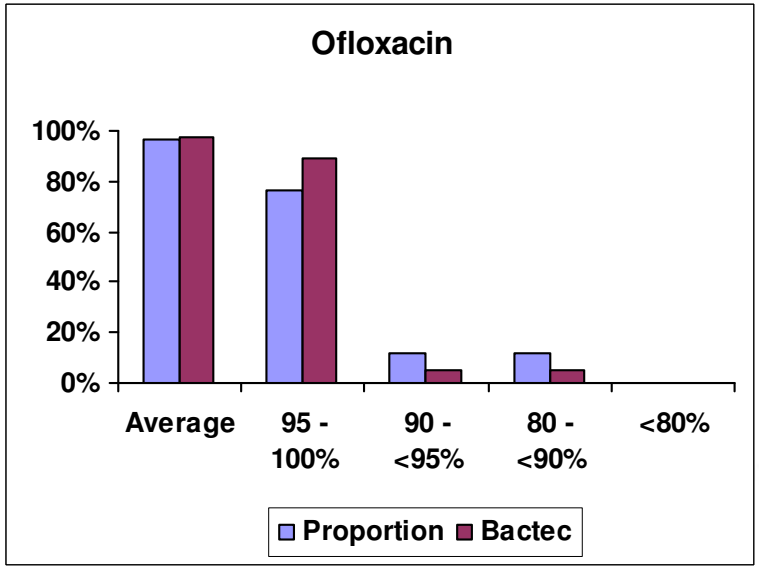
- First-line: shift towards MGIT
  - early rounds:
    - 70% LJ proportion
    - 13% BACTEC (radiometric or MGIT)
    - 1-2 agar / absolute concentration / resistance ratio
  - last round 36% LJ proportion, 46% MGIT
- Second-line: equal numbers LJ proportion / MGIT

Accuracy by method, Rd 14 to 17, average and distribution









Rd 17, critical concentrations

Drug	LJ	Agar	Bactec 460	MGIT 960
Kanamycin	30.0	5.0	4.0	(4.0)
Amikacin	40	(4.0)	1.0	1.0
Capreomycin	40	10.0	1.25	2.5
Ciprofloxacin	2.0	2.0	2.0	1.0
Ofloxacin	2.0	2.0	2.0	2.0

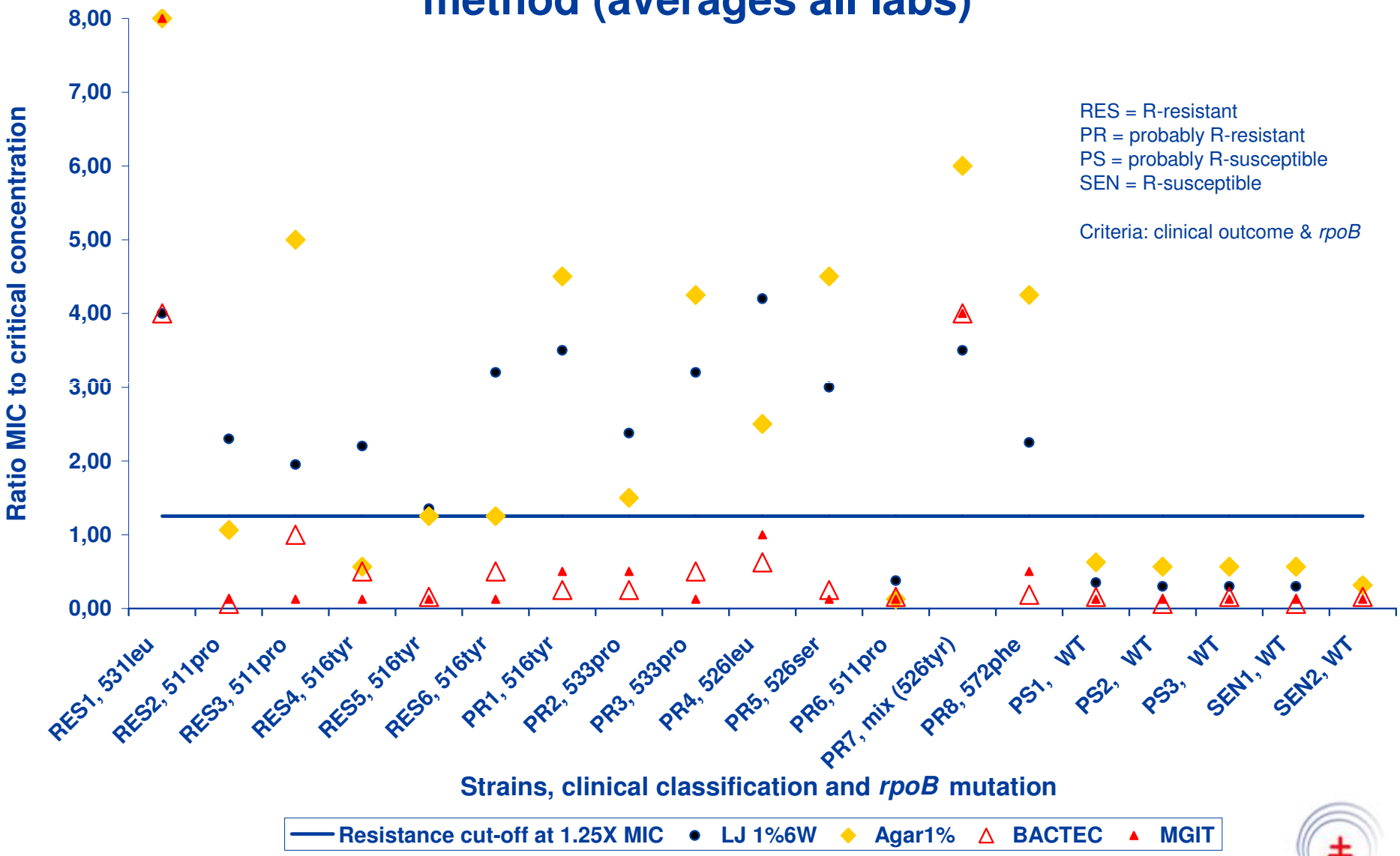
- Mostly ~ interim recommendations
  - Ak/Km in MGIT 1/4, but 4/3 in LJ
  - reason for MGIT Ak lower performance?

Problems encountered

- Strains yielding too low agreement (<80%)
  - low-level resistance: exists for all drugs?
  - inadequacy of judicial result criterion

    → strain excluded
  - average 10%: FLD results shown not quite realistic
    - e.g. RMP: 90-98% accuracy rather than 97-100% ?
  - partly linked to method used: RMP, EMB in MGIT
    - but also critical concentrations?
  - RMP investigation: linked to specific mutations

Borderline R-resistant strains, variation of MIC by method (averages all labs)



Rifampicin "borderline" resistance in the rounds

- Over-representation rare types / profiles
  - less in recent rounds
    - non-MDR subset dropped
    - re-use of highly concordant strains
  - but now said to be "easier than daily routine" ….

- Add clinical significance INH, RMP low-level resistance to resolve low agreement?
  - allow third result "intermediary resistant"
  - borderline INH still sufficiently active
    → intermediary or susceptible = correct
  - borderline RMP insufficiently active
    → intermediary or resistant = correct

Special round "borderline" strains

- Results from 10 volunteer SRL
- Very difficult panel of strains
  - 14 from coordinating SRL research RMP
    - "rare" *rpoB* mutations
    - mostly discordant MIC99 on LJ versus MGIT
  - 6 from rounds, all but 1 INH discordant
- All methods or combinations allowed; variations of technique encouraged

Special round "borderline strains", SRLN 2011

STRAIN CODE	MIC determinations & MGIT pre-testing				Mutations
	INH_MIC	RMP_MIC	RMP_MGIT1	RMP_MGIT0.5	*rpoB*
1	NA	640 (LJ)	S	R	511Pro
2	NA	640 (LJ)	S	S	511Pro
3	NA	320 (LJ)	S	S	515Thr&516Gly
4	NA	80 (LJ)	S	S	526Asn
5	NA	320 (LJ)	S	S	516Tyr
6	NA	160 (LJ)	FAILED	FAILED	533Pro
7	NA	160 (LJ)	S	S	533Pro
8	NA	<=20 (LJ)	S	S	535Ser
9	NA	80 (LJ)	S	S	572Phe
~~10~~	~~NA~~	~~40 (LJ)~~	~~S~~	~~S~~	~~632Leu~~
11	>4 (agar)	<0.25 (agar)	NA	NA	Wildtype
12	NA	>640 (LJ)	R	R	511Arg&516Gly
13	NA	>640 (LJ)	R	R	516Val
14	NA	>640 (LJ)	FAILED	FAILED	526Asp
15	NA	>640 (LJ)	FAILED	FAILED	526Asp
16	0.5 (agar)	>120 (LJ)	NA	NA	526Asp
17	0.2 (agar)	>120 (LJ)	NA	NA	531Leu
18	0.5 (agar)	0.5 (agar)	NA	NA	Wildtype
19	0.2 (agar)	>120 (LJ)	NA	NA	531Leu
20	0.5 (agar)	80 (LJ)	NA	NA	516Tyr&515Ile

Total participating labs: 10

	Method used:	No. of labs
1*	Proportion method LJ	2
2*	Proportion method agar	1
3*	Absolute conc. LJ	1
4*	Absolute conc. agar	1
6*	MGIT	5

ISONIAZID

	No. of labs with results in the range of					Average
	100%	95-99%	90-94%	80-89%	<80%	score
SENSITIVITY	8	0	1	1	0	98%
SPECIFICITY	2	0	0	1	7	54%
PREDICTIVE VALUE RESISTANT	2	0	1	3	4	80%
PREDICTIVE VALUE SUSCEPTIBLE	6	0	0	1	1	93%
EFFICIENCY	1	2	1	3	3	83%

RIFAMPICIN

	No. of labs with results in the range of					Average
	100%	95-99%	90-94%	80-89%	<80%	score
SENSITIVITY	0	0	4	1	5	76%
SPECIFICITY	8	0	0	0	2	95%
PREDICTIVE VALUE RESISTANT	8	0	2	0	0	98%
PREDICTIVE VALUE SUSCEPTIBLE	0	0	0	2	8	50%
EFFICIENCY	0	2	3	0	5	80%

Discussion special round

- "Intermediate" results
  - INH 5%, RMP 10% of reported: not misused
  - but frequent from evaluation: 35% INH, 40% RMP
- All knew genotypic result
  - but only 1 SRL made it overriding for RMP
- Methods / variations used
  - best results: absolute conc. + wide range intermediate
  - lowest scores: MGIT users
  - lowering critical concentrations, extending incubation: helpful but not sufficient

But are these borderline strains important??

- Investigations Antwerp SRL: RMP
  - frequency?
  - prognostic value?
  - proportion missed phenotypic DST?
- Answer: yes, they are important
  - 10-20% of all *rpoB* mutations; more with early DST
  - same, unfavourable prognosis (more relapse)
  - some systematically missed by MGIT: 511pro, 533pro…
    - or by all rapid DST?
- Controversy geno- / phenotypic DST !

Frequency of rare rpoB mutations, without pre-selection based on culture / phenotypic DST

	Hong Kong, unselected	Kinshasa, first failures and relapses	Bangladesh, all retreatment cases
Total with rpoB mutation, N	73	453	320
511Pro	7	1	13
516Tyr		11	10
526Cys	1		1
526Leu	3	11	14
526Asn		10	3
533Pro		9	16
535Ser		3	
572Phe		6	1
Del509-511		2	
Low-level RMP-resistance mutations, %	15%	12%	18%

First-line treatment outcome versus rpoB mutation

	Episodes	Outcome, percents		
Mutation	N	Unfavourable	Failure or relapse	Ratio failure/relapse
	"High-level" R-resistance			
513Glu/Lys/Pro	4	75	75	All failures
516Phe/Val	18	100	83	4
526Arg/Asp/Tyr	51	78	60	5.2
531Leu/Trp	155	83	65	6.1
	"Borderline" R-resistance			
511Pro	15	90	75	1.1
516Tyr	7	71	57	All failures
526Asn/Cys/Leu	16	75	69	4.5
533Pro	18	67	61	1.8
572Phe	5	60	40	All failures
	Uncertain level R-resistance			
522Leu	3	100	100	All failures
DEL/INS512_513	7	86	86	1

Conclusions and way forward

Rounds achievements

- Standardised use of DST (first-line)
  - initial discordance++: soon resolved through uniformisation of techniques
  - less consistent for EMB, SM: cut-off?

- But:
  - remains too much centered on SRL
  - findings should be used to improve techniques
    - rather than excluding difficult strains

Panels dissemination

- Insufficiently passed on to NRLs
  - particularly poor coverage: Africa
  - safety & transport problems (JATA; imports)

- Wider coverage by coordinating SRL?
  - could be more efficient to cover also NRL
  - still a need for further distribution
    - but sub-national less problematic for transport from NRL

Borderline strains RMP, INH

- Proposed to stop their exclusion
  - during pre-screening; during analysis
  - panel composition targeting 50% resistance
    - priority to INH, RMP, Km, Ofx
    - range of profiles ~ early retreatment cases
  - protocol as for special round: allow “intermediate”
    - Km, Ofx evaluation as for INH ?