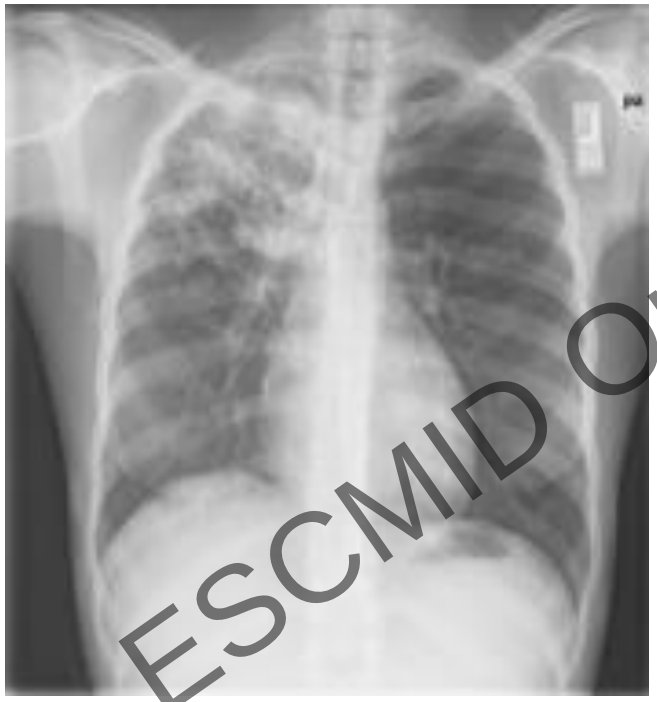
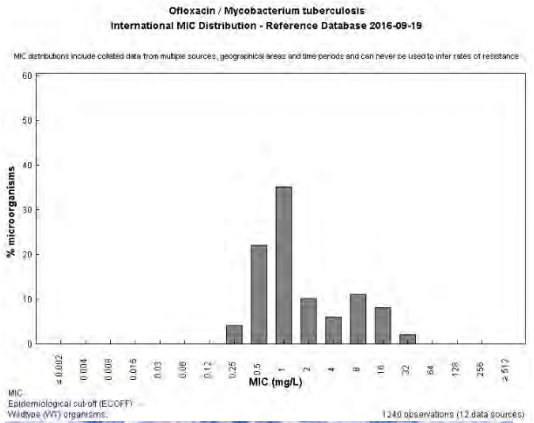
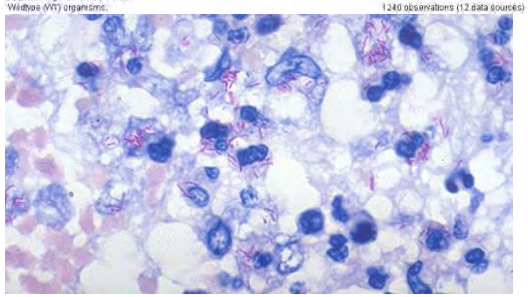
The role of MIC determinations and *M. tuberculosis* subpopulations - what would be clinically useful?







Thomas Schön MD PhD

Senior consultant in Infectious Diseases and Clinical Microbiology; Kalmar County Hospital. Associate professor, Linköping University

MIC determinations – why ?

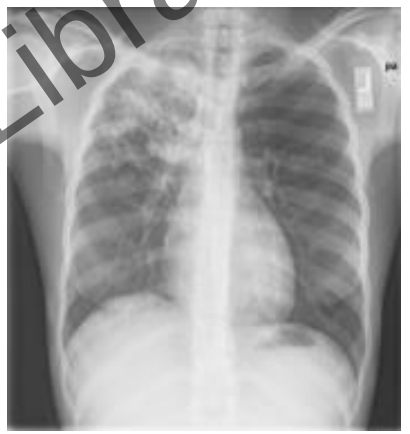
- Therapeutic drug monitoring (TDM)
- Low level resistance
- Establish drug susceptibility breakpoints

Van der Burgt, Alffenaar et al. ERJ, 2015; Peloquin, CA et al. Drugs, 2014; Ängeby, Schön et al. Bull WHO, 2012

Individualized treatment

MIC (susceptiblity level)
Resistance mutations

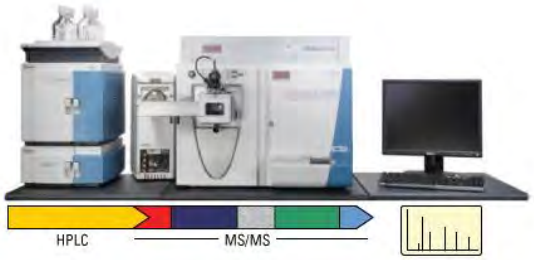
Host
Immune
defence



Clinical evaluation - TB-score
Patient factors

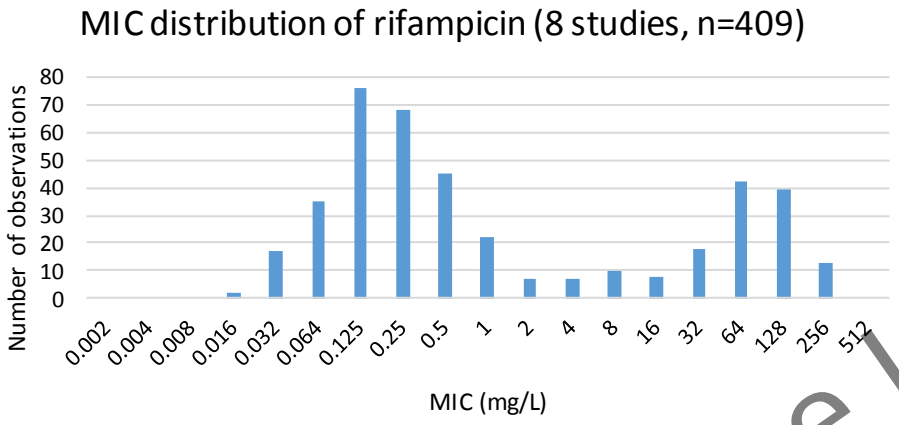
TDM

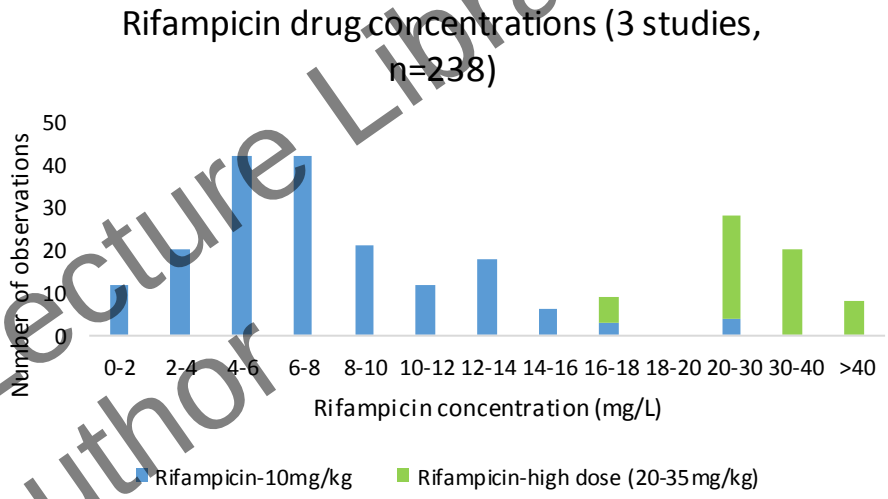
Drug concentrations
LC-MS/MS

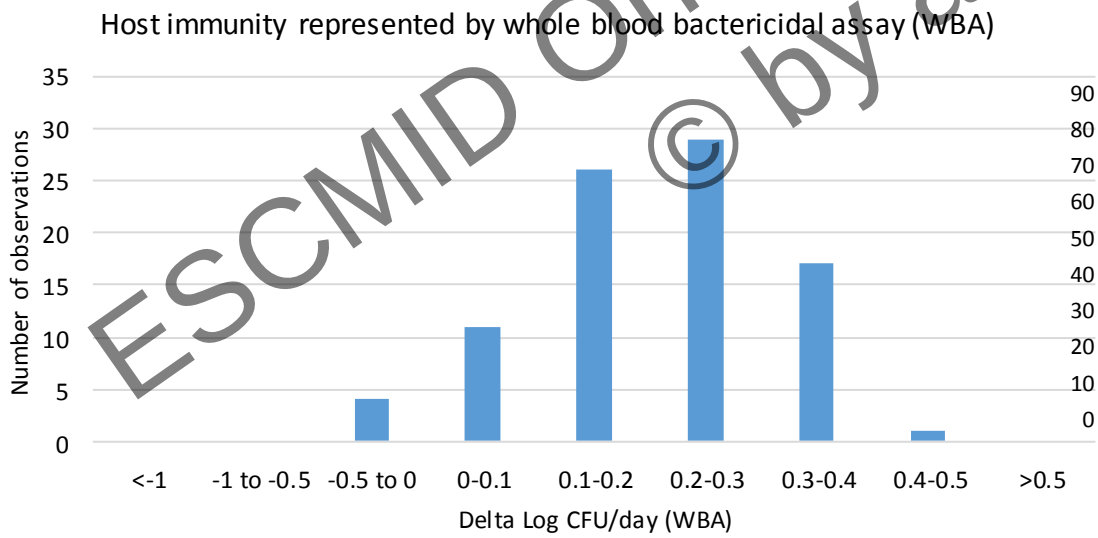


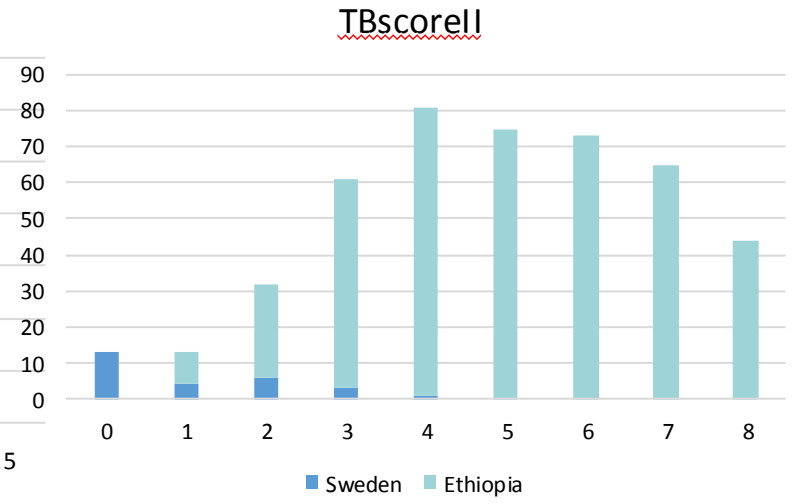
Bacterial load – CFU/time to positivity (TTP)

TDM/MIC in clinical practice – Rifampicin





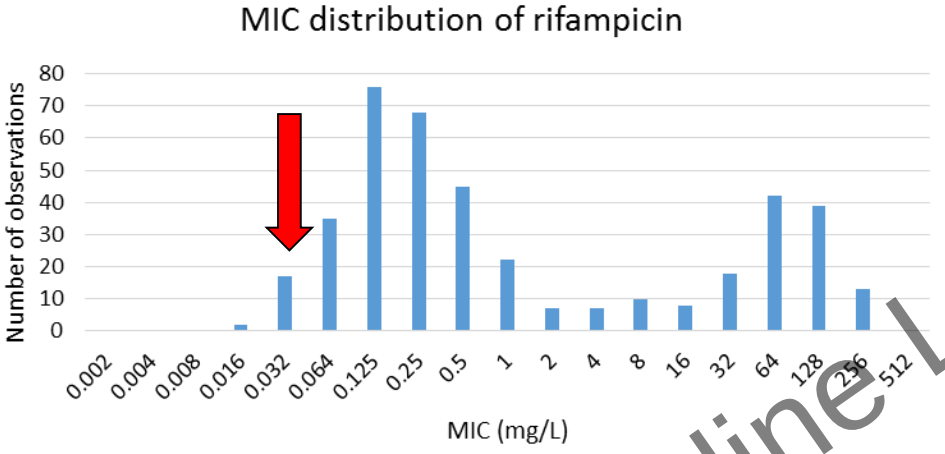


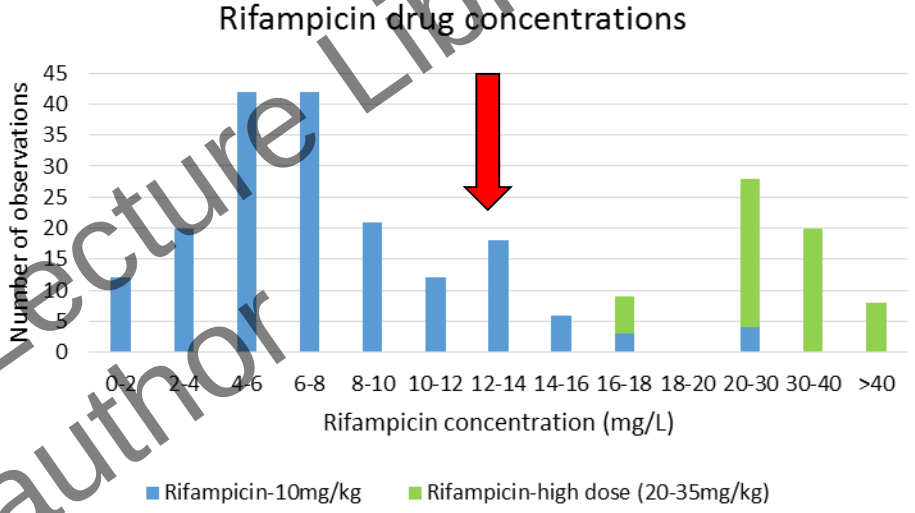


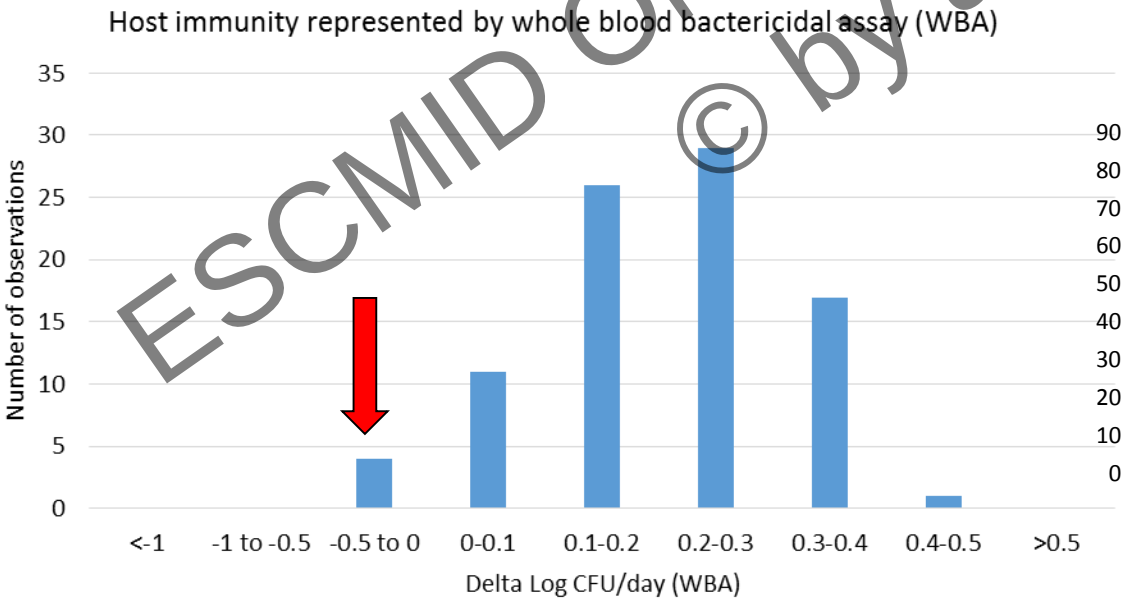
Gumbo et al, J Inf Dis 2013, Boeree et al, Am J Respir Crit Care Med 2015, Rudolf, Schön, Wejse et al SJID 2013
Wallis et al, J Inf Dis 2010+2003, Schön et al, J Antimcrob Chemother 2009+2013

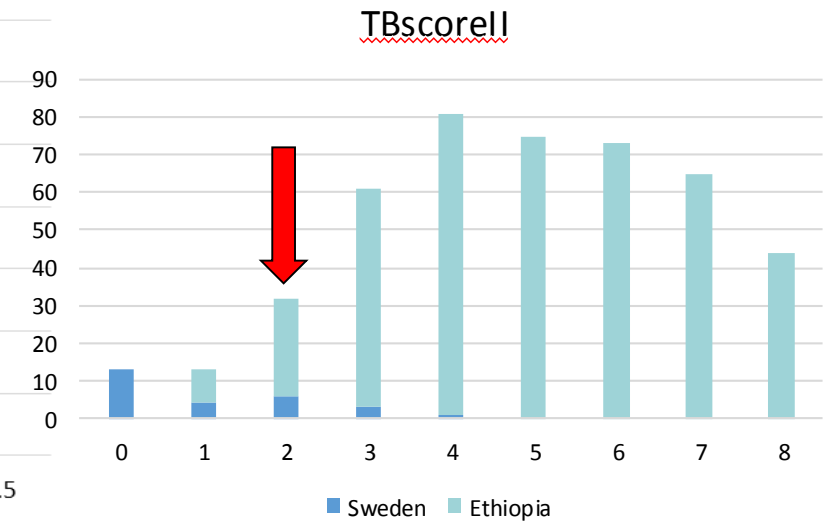
Case 1. Drug susceptible pulmonary TB (600mg RIF)

MIC: 0.032 mg/L, Rif serum conc: 12.7 mg/L WBA -0.24, TB-scoreII:2



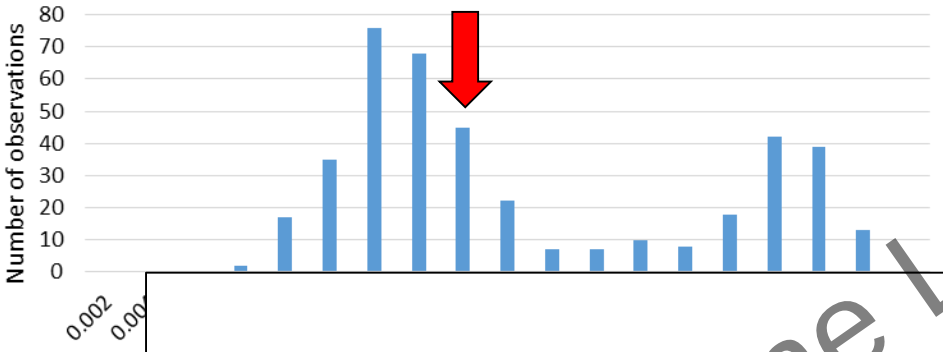


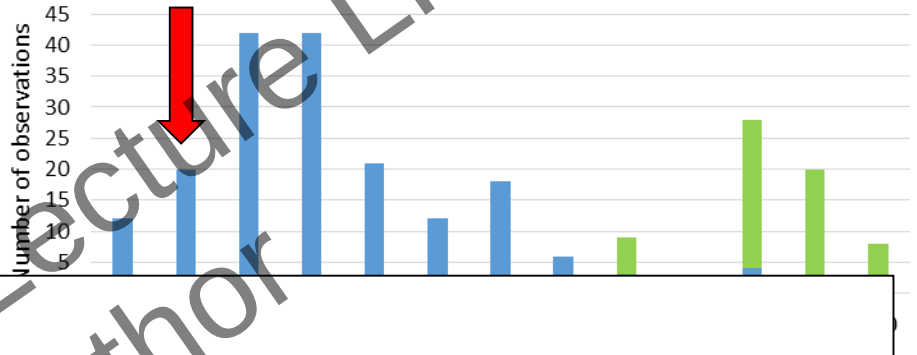




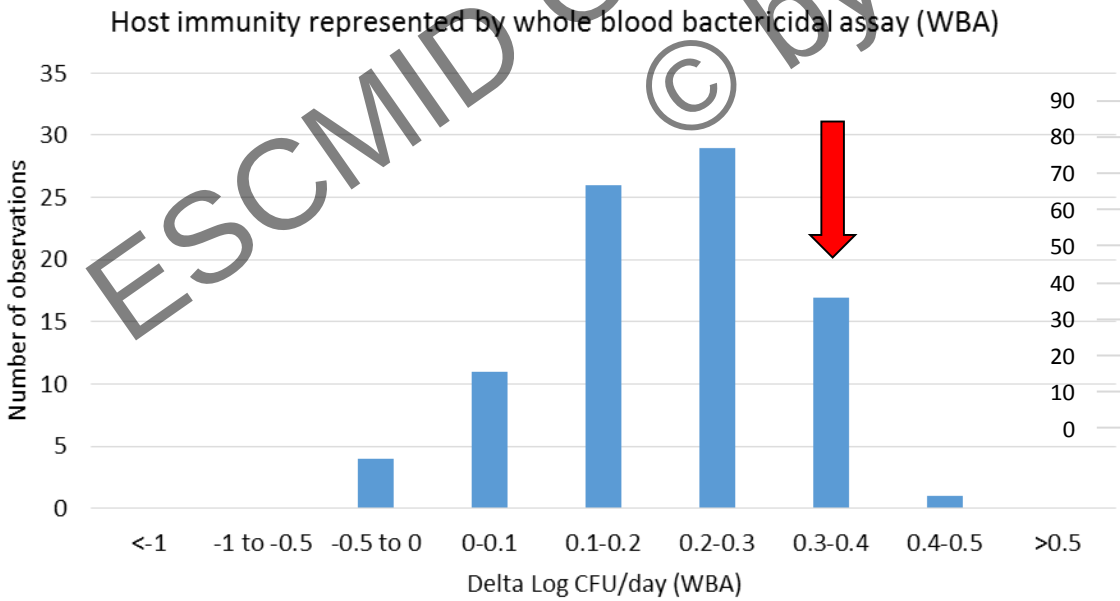
Case 2. Drug susceptible pulmonary TB (600mg RIF)

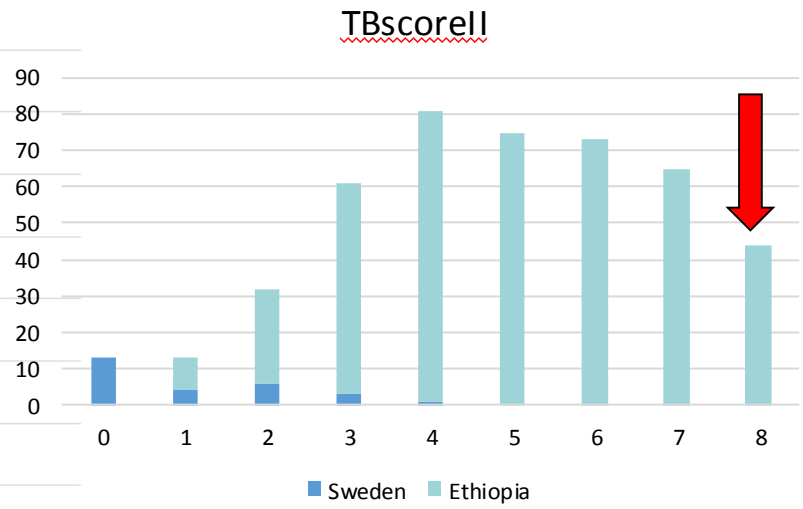
MIC: 0.5 mg/L, Rif conc: 3.1 mg/L, WBA: 0.32 CFU/day, TB-scoreII:8





1. **The RIF dose needed for cure is dependent on the MIC**
2. **Could higher RIF dosing vs MIC lead to shorter regimens ?**





Gumbo T, et al. AAC 2014.

MIC and Low level resistance

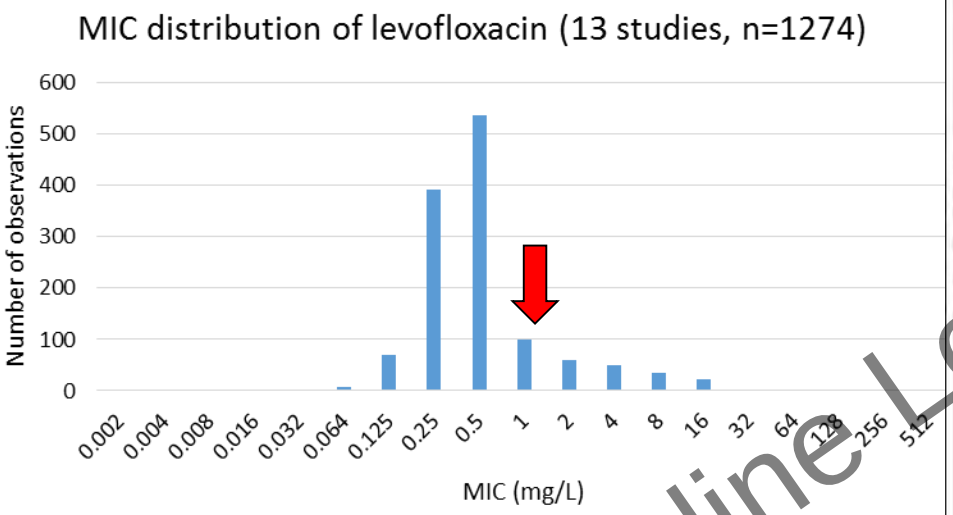
Action	Mutation	MIC range	DST	Hain	Frequency (%)	Clinical outcome data
HD FQ?	FQ *gyrA* A90V	LEV 0.5-2 MOX 0.25-2	S/R(I)	*gyrA* Mut 1	20-30	+/-
HD AMI/CAP?	AK/CAP: *Eis* C-12T, C-14T G-10A	KAN 8-32 AMI 1-4 CAP 2-8	KAN R AMI S/R(I) CAP S/R(I)	*Eis* Mut 1 (WT-)	5-22	-
HD INH?	INH: *inhA* C-15T A-16G T-8C/A	INH 0.25-2	INH R(I)	*inhA* Mut1 Mut2 Mut3ab	10-20	+/-
HD RIF?	RIF: *rpoB* D516Y L533P H526S/L	RIF:0.25-8	RIF S/R(I)	*rpoB* WT3/4- WT8- WT7-	<0.1	+/-
HD RIB?	RIF: *rpoB* D516V	RIF:2-64 RIB:0.064-0.5	RIF R RIB S/R(I)	*rpoB* Mut 1	5-32	+/-

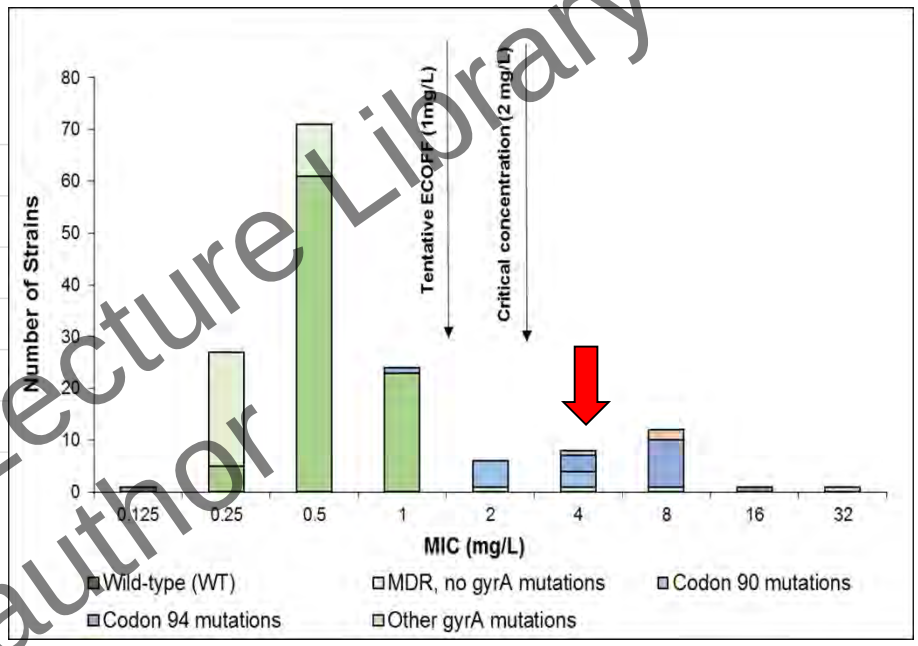
Domınguez, Lange et al, IJTLD 2016; Tagliani, et al. JCM 2015, Brosier et al. JCM 2016. Rigouts L, et al. JAC 2015
Cambau JAC 2015; Ängeby, Schön, Niward et al, J Antimcrob Chemother 2010+2016

Low level resistance – mutations vs MICs

- Clinical outcome data limited
  - *inhA*, *gyrA* codon 90, *rpoB* A516V, *eis, embB* 306
  - Intermediate (I) - dose increase - TDM
- Each resistance mutation - MIC distribution
  - MIC determination if "low level resistance mutation"
- Are critical concentrations = clinical breakpoints = gold standard ?

Pre-XDR or not ?





MDR; OFL R, LEV S, MOX S
Dose 750mgx1
Cmax:11.2 mg/L
LEV MIC 1mg/L, OFL MIC 4mg/L
HAIN *gyrA* mut 1 (A90V), WGS:A90V

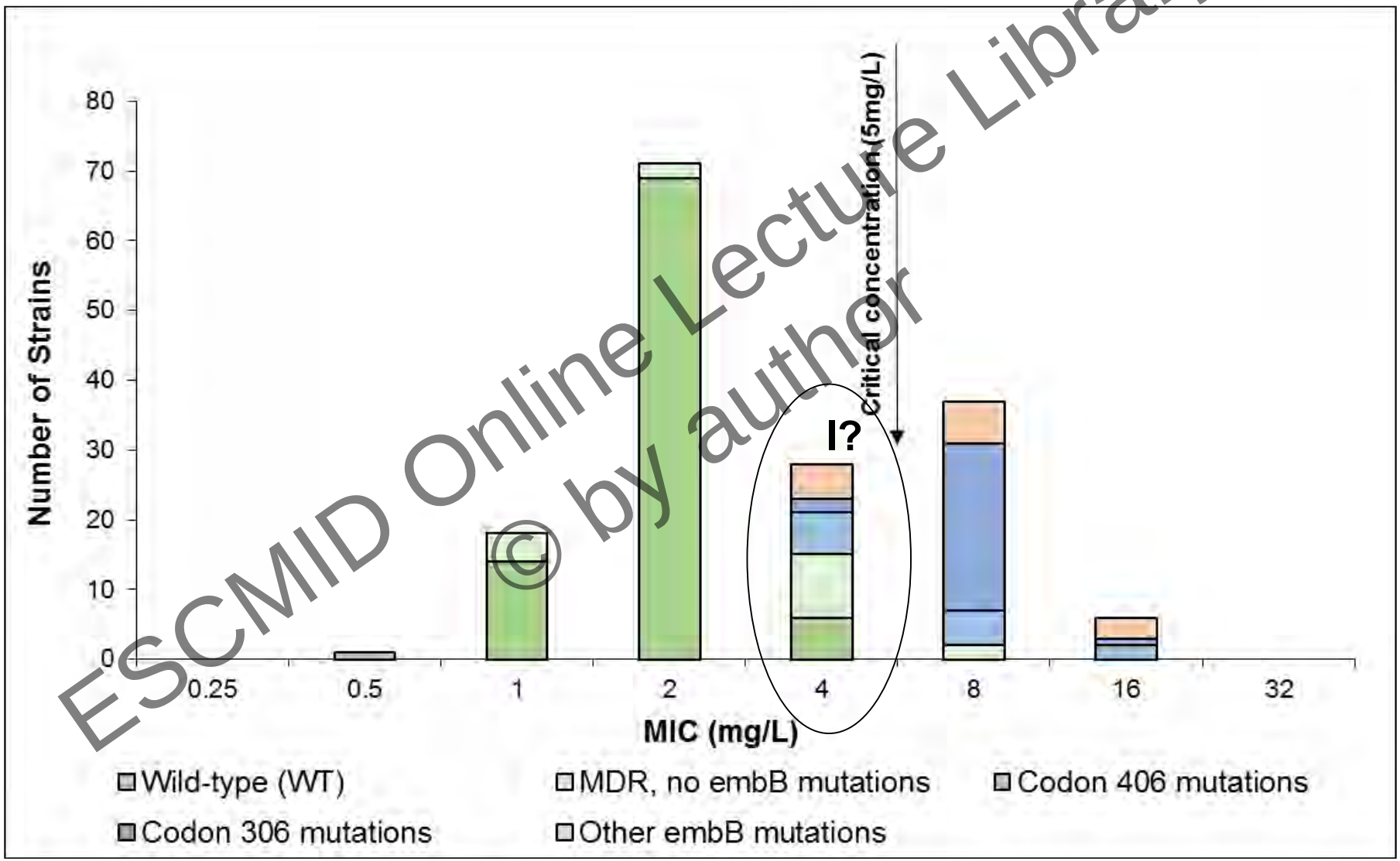
Ghimire, Alffenaar et al. JAC, 2016; Ängeby, Schön, Niward et al, J Antimcrob Chemother 2010+2016

MICs and clinical breakpoints

- Can we be sure about the gold standard ?
  - EUCAST: PK/PD, clinical outcome, MIC
  - FQs, CAP, RIB, ETH/PTO, EMB?
- Methodology – MIC determination
  - No reference standard (!)
  - Cathegorical S/R validation of new methods (?)
    - LJ->7H10->B460->MGIT->TREK->…
  - MIC distributions for EUCAST
- Quality control

Kahlmeter G. JAC 2015; Ängeby, Schön, Niward et al, JAC 2009+2016

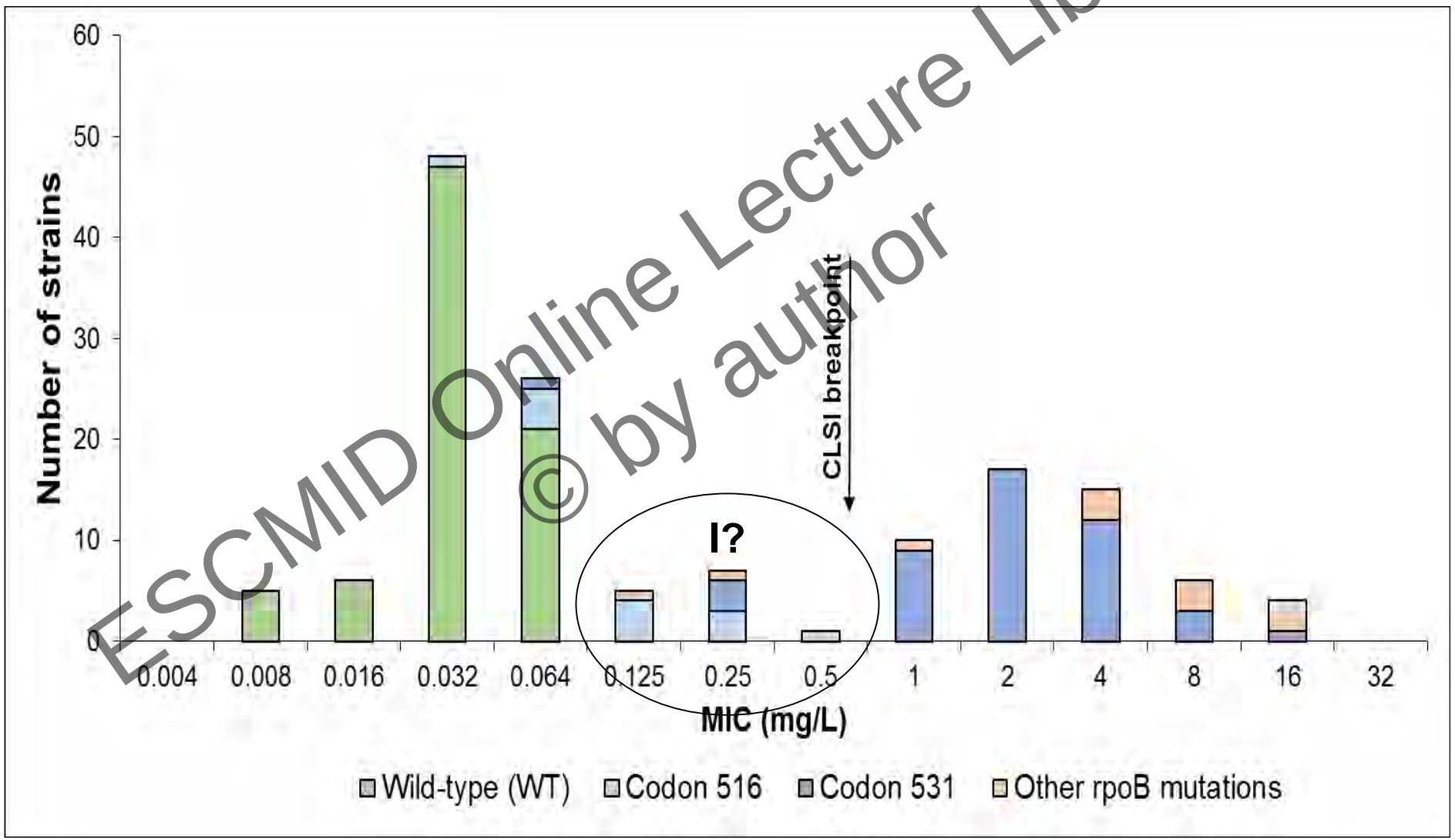
Ethambutol



Schön T et al JAC 2009
and unpublished observations

		WHO (2013/1998)	CLSI (2011)	WHO
	LJ	7H10	7H10	MGIT
EMB	2	5	5/10	5

Rifabutin (RIB S, RIF R ?)



Schön T. et al. JAC. 2013

Standard media for MIC testing of *M. tuberculosis*

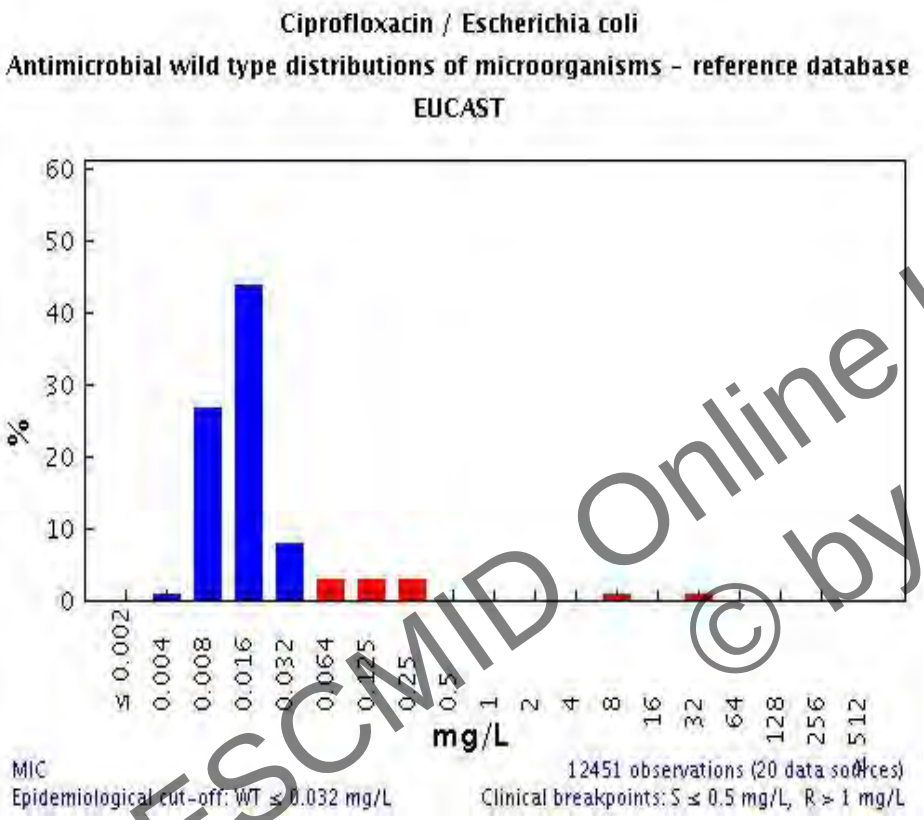
- Löwenstein Jensen (LJ)
  - Pro: Widely used, inexpensive
  - Con: Variation in preparation, takes time (4v)
- Middlebrook 7H9 (used in TREK/MGIT)
  - Pro: 96-well format possible
  - Con: Contamination, ”readability” (TREK)
- Middlebrook 7H10/7H11
  - Pro: Used by CLSI (standardized protocol)
  - Con:Variation in preparation, contamination

WHO, ECDC, CLSI guidelines.

ECOFFs vs Clinical breakpoints

- ECOFF (epidemiological cut-off):
  - Highest MIC of organisms lacking phenotypically expressed resistance
  - The lowest possible breakpoint
  - A tool in determining clinical breakpoints
  - Sensitive detection/surveillance of resistance
- Clinical breakpoints:
  - MIC-concentrations decided by man to separate treatable from non-treatable organisms
  - Based on ECOFFs, PK/PD- and clinical outcome data
  - Predict outcome (SIR-system)

What is a wild type distribution ?



- EUCAST: Gaussian MIC distribution for organisms without resistance mechanisms

- Establishing a clinical breakpoint (SIR)
  - Wild-type MIC distributions (ECOFF)
  - PK/PD simulations
  - Clinical outcome data

- ECOFF is NOT= clinical breakpoint
  …nor the critical concentration:

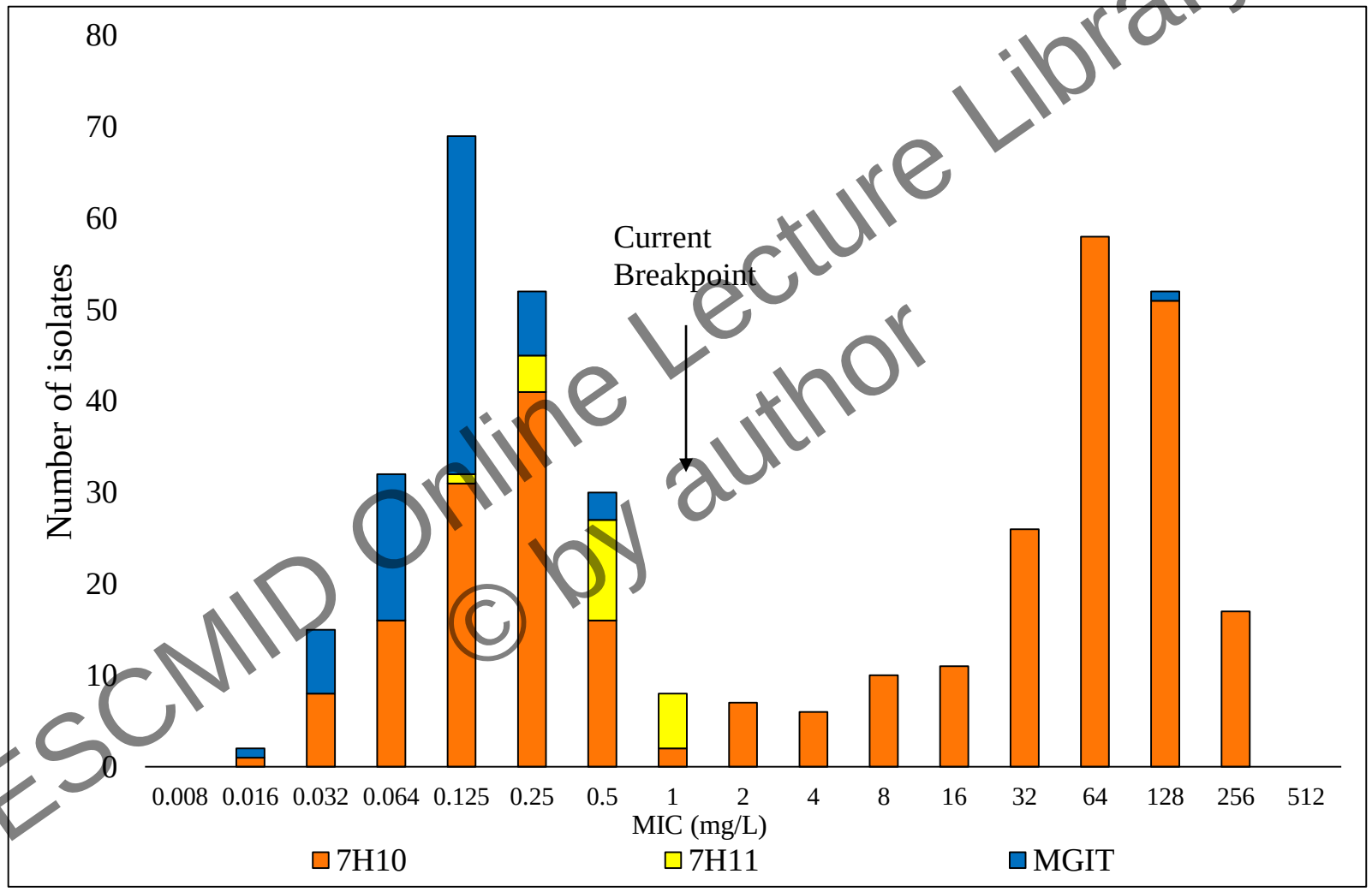
"…the lowest concentration of drug that will inhibit 95% (90% for pyrazinamide) of wild strains of M. tuberculosis that have never been exposed to drugs, while at the same time not inhibiting clinical strains of M. tuberculosis that are considered to be resistant (e.g. from patients who are not responding to therapy)".

Kahlmeter J Antimicrob Chemother. 2015 Sep;70(9):2427-39, Canetti et al, Bull WHO 1963.

MIC distributions for EUCAST

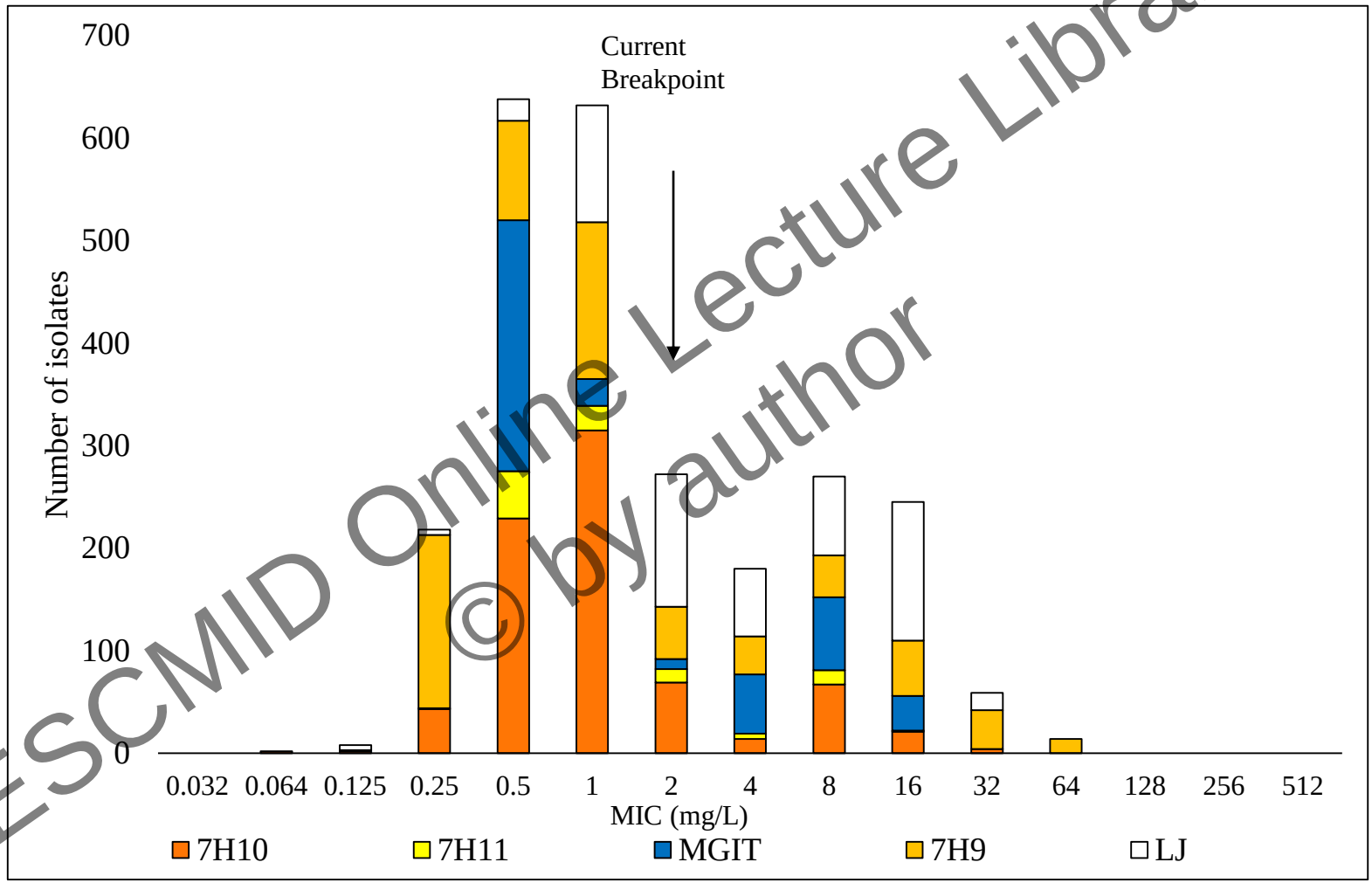
- Literature study
  - Truncation and non-standard dilutions
- http://mic.eucast.org/Eucast2
- Standard methods (7H9/10/11, LJ, MGIT)
  - Not colorimetric methods, QC
- Compared to other pathogens
  - Bias towards inclusion of MDR/XDR isolates

Rifampicin MIC-distribution



	LJ	WHO (2013/1998) 7H10	CLSI (2011) 7H10	WHO MGIT
RIF	40	1	1	1

Ofloxacin MIC-distribution



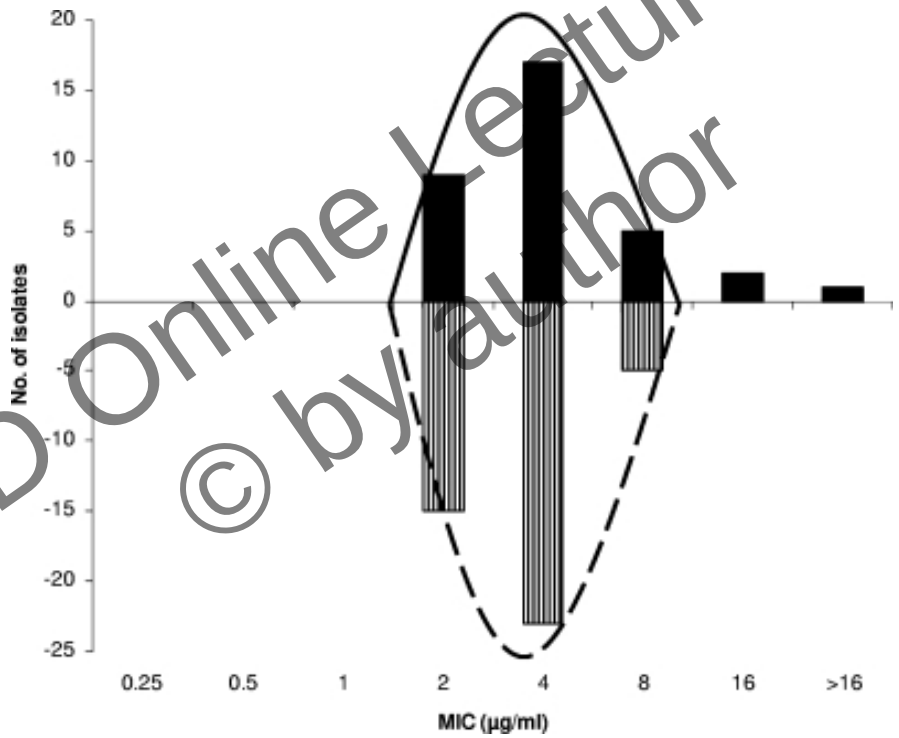
		WHO (2013/1998)	CLSI (2011)	WHO
	LJ	7H10	7H10	MGIT
OFL	4 (2)	2	2	2

Quality control in MIC determination

- Reference MIC-ranges for H37Rv
  - Used for QC in DST of bacterial pathogens
  - Reproducibility – NOT S/R only !
- Proficiency testing
  - Not only S/R
  - H37Rv and isolates close to Bp (MOX/OFL, EMB, RIF)

What is the reproducibility of your comparator DST method?

- ISO 20776-2: within ± 1 dilution of the mode for ≥95 % of the results
- *Usually below* ± 1 dilution



Fluconazole MIC distributions of 34 individual *Candida glabrata* isolates (black bars) compared with the MICs obtained by 51 repeated tests of a single *C. glabrata* isolate (striped bars) originally determined to have a MIC of 2 µg/ml.

ISO 20776-2

Arendrup et al. AAC2009

Summary – MIC determinations

- TDM - quantify resistance
- Phenotype – genotype correlation
- Establishing clinical breakpoints