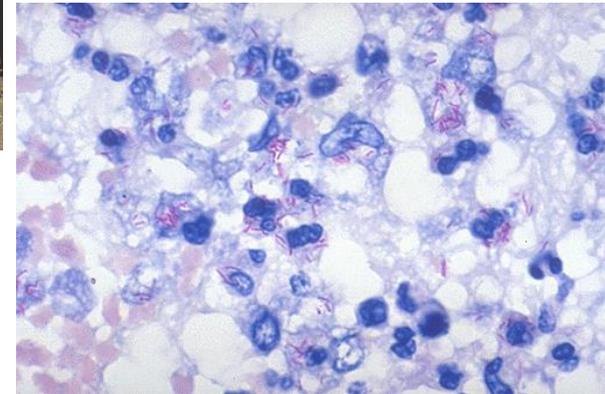
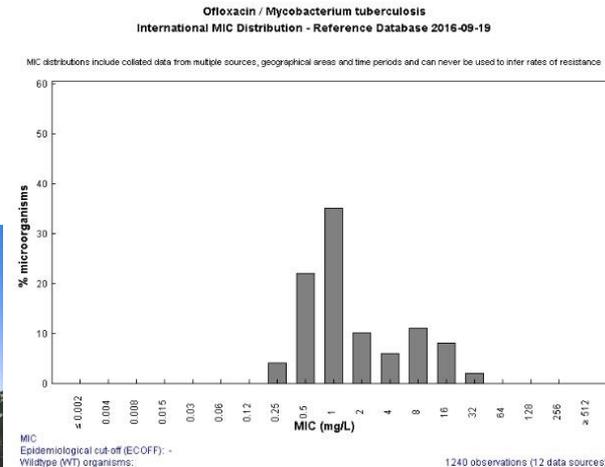


Susceptibility testing of mycobacteria



Thomas Schön MD PhD

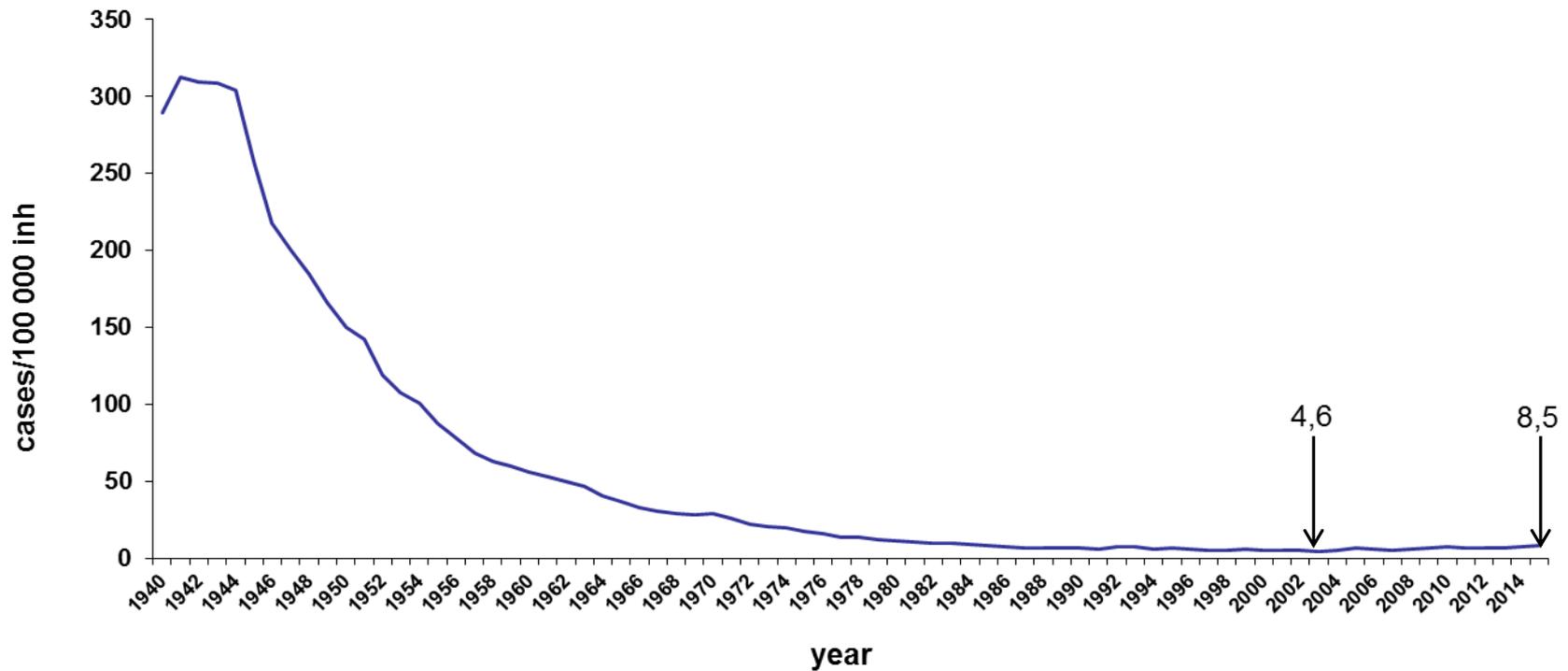
Senior consultant in Clinical Microbiology and Infectious Diseases;
Kalmar County Hospital. Associate professor, Linköping University

What is special about *M. tuberculosis* AST ?

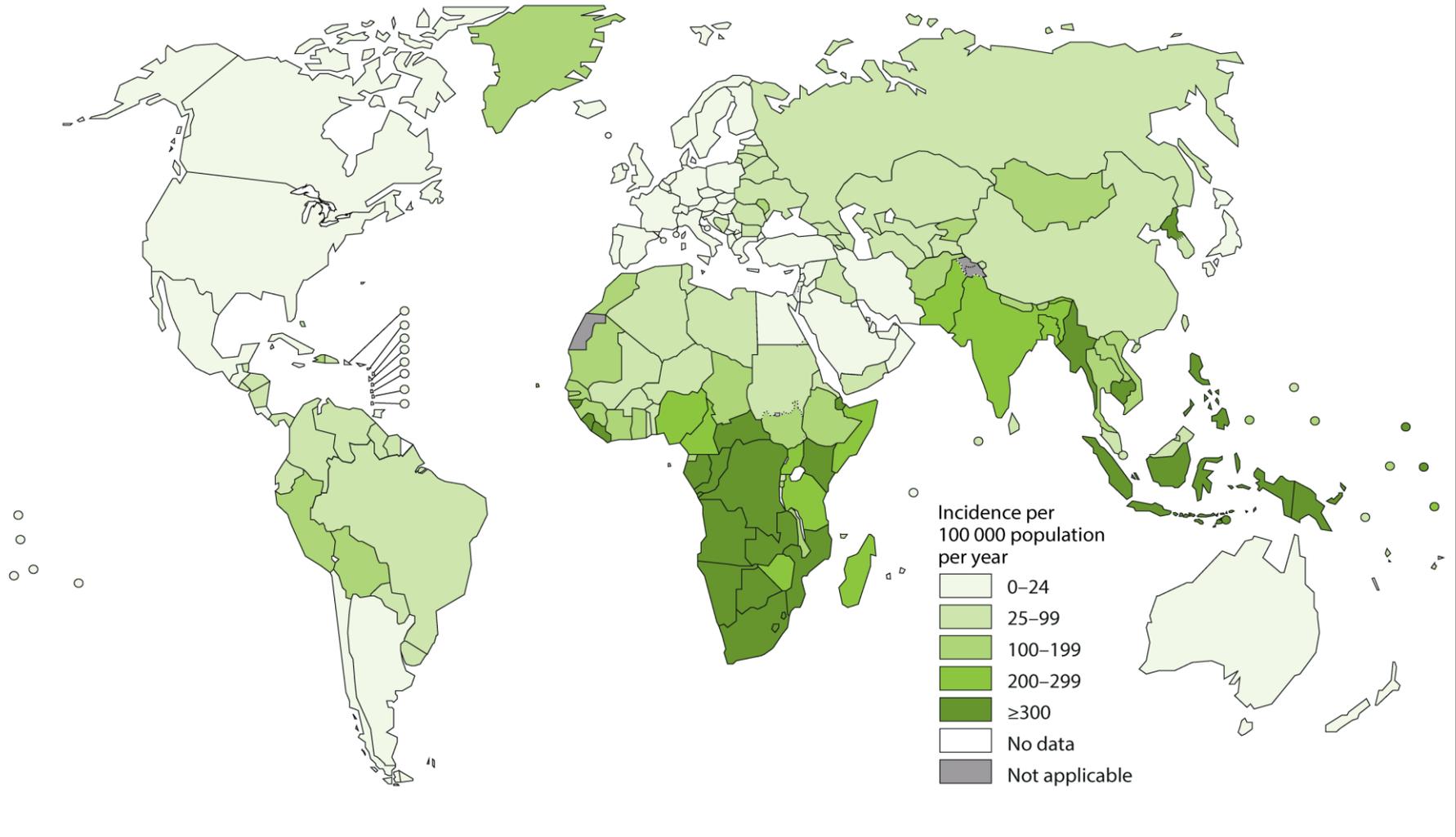
	<i>E coli</i> (rapid growers)	<i>M. tuberculosis</i>
Duplication time	10-20 min	12-18h
SIR (clinical breakpoints)	Yes	No (except DLM and BDQ)
ECOFFs defined	Yes	No (not enough data)
Disc diffusion (MICs) used in clinical practice	Yes	No (S/R testing at single concentrations in >4 different media using 3 strategies)
Time to culture/AST	1-24h/4-24h (BSL2)	>1-2 weeks/1-2 weeks (BSL3)
Molecular tests used to rule in resistance/MDR	Not routinely	Yes (Xpert MTB/RIF, HAIN etc)
Usual treatment strategy	Single therapy	Multi drug regimens (>4 drugs)
Treatment duration	Days-weeks (colonisation)	Always treated. Minimum 6 months

Transition to low-incidence

TB-incidence in Sweden 1940 - 2015



Estimated TB incidence rates, 2016



The boundaries and names shown and the designations used on this map do not imply the expression of any opinion whatsoever on the part of the World Health Organization concerning the legal status of any country, territory, city or area or of its authorities, or concerning the delimitation of its frontiers or boundaries. Dotted and dashed lines on maps represent approximate border lines for which there may not yet be full agreement.

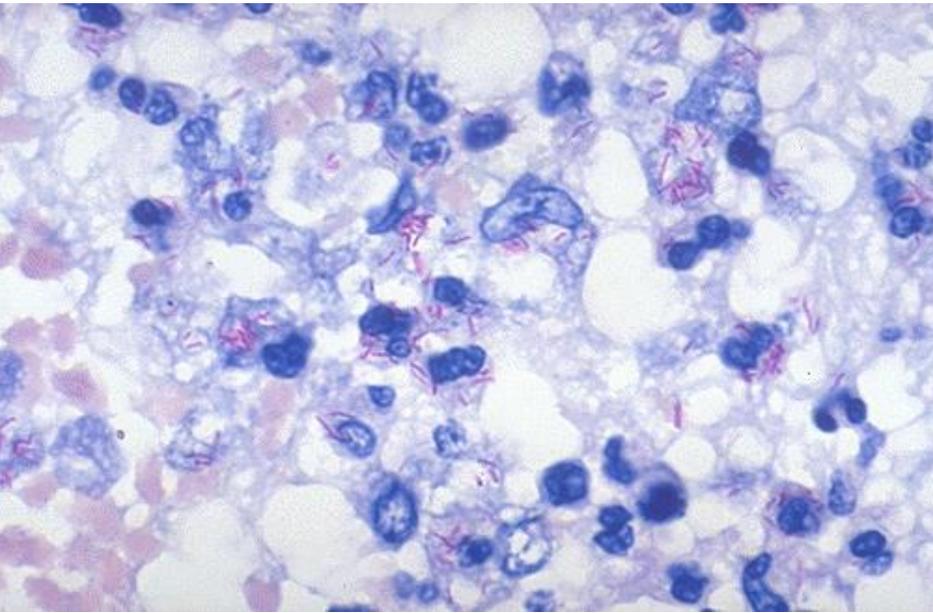
Data Source: *Global Tuberculosis Report 2017*. WHO, 2017.

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What is tuberculosis?

- *Mycobacterium tuberculosis*
- Several outcomes of exposure !
- Pulmonary disease (>60%)
- Symptoms:
 - **Cough**, weight loss, night sweating, neck swelling



Standard treatment of TB

- Isoniazid 300 mg, 6m
- Rifampicin 600 mg, 6m
- Pyrazinamide 1,5 g (20 mg/kg), 2m
- Ethambutol 1,2 g (15mg/kg), 2m
- Pyridoxine 40 mg, 6m

-> **Usually, TB treatment is straight forward**

**1st
Line**

Rifampicin	Isoniazid
Ethambutol	Pyrazinamide

Resistance to:

RIF + INH = MDR-TB

**2nd
Line**

Fluoroquinolones <i>Levofloxacin, moxifloxacin, ofloxacin</i>
Aminoglycosides <i>Kanamycin, amikacin, capreomycin</i>

**1st
Line**

Rifampicin	Isoniazid
Ethambutol	Pyrazinamide

Resistance to:
RIF + INH = MDR-TB

**2nd
Line**

Fluoroquinolones <i>Levofloxacin, moxifloxacin, ofloxacin</i>
Injectables <i>Kanamycin, amikacin, capreomycin</i>

Any fluoroquinolone
+
Any injectable

= XDR-TB

Table 6. Medicines recommended for the treatment of rifampicin-resistant and multidrug-resistant TB¹

A. Fluoroquinolones²	Levofloxacin Moxifloxacin Gatifloxacin		Lfx Mfx Gfx
B. Second-line injectable agents	Amikacin Capreomycin Kanamycin (Streptomycin) ³		Am Cm Km (S)
C. Other core second-line agents²	Ethionamide / Prothionamide Cycloserine / Terizidone Linezolid Clofazimine		Eto / Pto Cs / Trd Lzd Cfz
D. Add-on agents (not part of the core MDR-TB regimen)	D1	Pyrazinamide Ethambutol High-dose isoniazid	Z E H ^h
	D2	Bedaquiline Delamanid	Bdq Dlm
	D3	<i>p</i> -aminosalicylic acid Imipenem-cilastatin ⁴ Meropenem ⁴ Amoxicillin-clavulanate ⁴ (Thioacetazone) ⁵	PAS Ipm Mpm Amx-Cly (T)

¹ This regrouping is intended to guide the design of conventional regimens; for shorter regimens lasting 9-12 months the composition is usually standardised (See Section A)

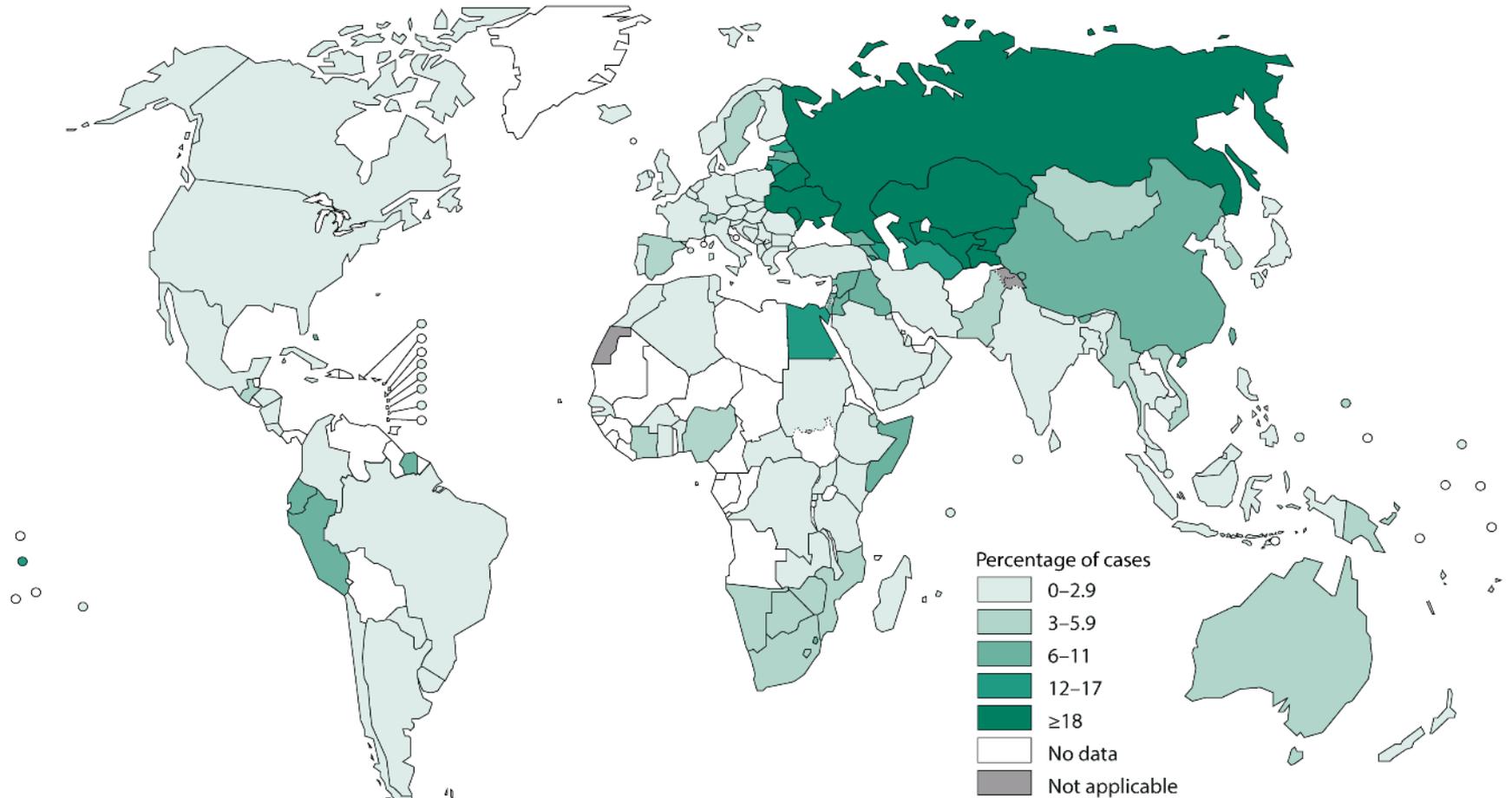
² Medicines in Groups A and C are shown by decreasing order of usual preference for use (subject to other considerations; see text)

³ Refer to the text for the conditions under which streptomycin may substitute other injectable agents. Resistance to streptomycin alone does not qualify for the definition of extensively drug-resistant TB (XDR-TB)(26)

⁴ Carbapenems and clavulanate are meant to be used together; clavulanate is only available in formulations combined with amoxicillin

⁵ HIV-status must be tested and confirmed to be negative before thioacetazone is started

Percentage of new TB cases with MDR/RR-TB



* MDR = multidrug-resistant ; RR= rifampicin-resistant
MDR/RR-TB = RR-TB cases including MDR-TB cases

Figures are based on the most recent year for which data have been reported, which varies among countries. Data reported before the year 2002 are not shown.

In total: ~ 500 000 MDR TB and ~50 000 XDR TB cases !
MDR TB, 15 % in previously treated and 3 % in new patients



Figure 9: Pill burden for patients with MDR tuberculosis

(A) Pill burden for a child aged 8 years. (B) Pill burden in an adult, including morning dose (left panel) and evening dose (right panel). These photos exclude any injectable agents, which are usually given in addition to pills, for 6–8 months intramuscularly without anaesthesia. Images with courtesy and permission of CAPRISA.

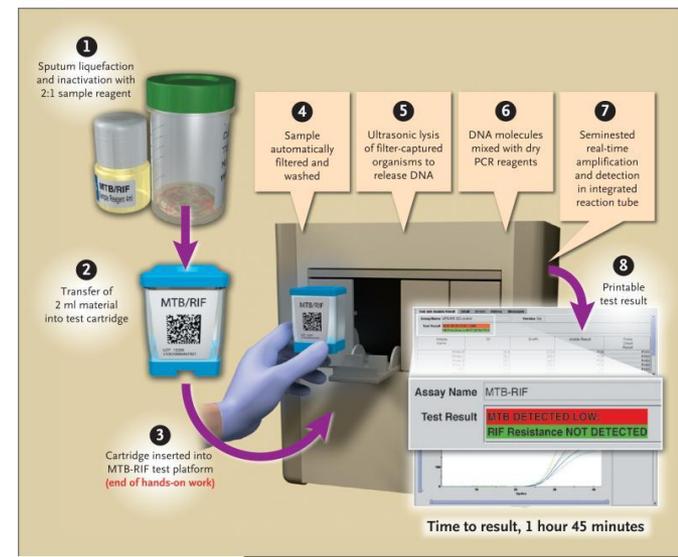
	Susceptible TB	MDR TB	XDR TB
Cost (Euro)	334 (40-1200)	23000 (2100-77000)	90000 (65000-150000)
Mortality	5%	10-40%	50-70%

Development of drug resistant TB

- Natural resistance
 - Thick cell wall, slow generation time
- Random mutations – not horizontal transfer
- **Selection during suboptimal therapy → transmission !**
 - MDR/XDR TB associated to previous treatment
 - 1940ies monotherapy
- Risk factors for XDR/MDR
 - Contact history and/or poor compliance

What's new in diagnosis and treatment of TB ?

- Shorter treatment duration of MDR TB
- Bedaquiline and Delamanid
- Personalized medicine
 - MIC determination, drug concentrations
- Rapid tests – drug resistance mutations
 - HAIN, Xpert MTB/RIF Ultra
- WGS directly on sputum samples
 - Rapid prediction of S and R !



Standard media for susceptibility testing of *M. tuberculosis*

- Löwenstein Jensen (LJ)

- Pro: Widely used, inexpensive
- Con: Variation in preparation, takes time (4w)



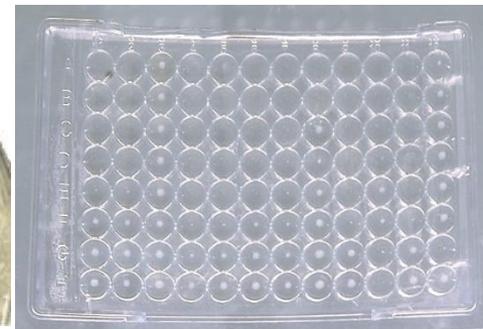
- Middlebrook 7H9 (used in MYCOTB/MGIT)

- Pro: 96-well format possible, established (MGIT), "rapid"
- Con: Contamination, "readability" (MYCOTB)



- Middlebrook 7H10/7H11

- Pro: Used by CLSI (standardized protocol)
- Con: Variation in preparation, contamination



Which are the challenges in susceptibility testing of mycobacteria ?

- Slow (phenotypic testing) and resource demanding (P3)
- Many methods and strategies
 - WHO ("critical concentrations"), no standard method!
 - Indirect proportion method: 1:100 diluted control without antibiotics
- Unclear definitions - "critical concentrations"
 - What about WT-distributions, PK/PD and clinical outcome ?
 - QC-ranges not defined

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journal homepage: www.clinicalmicrobiologyandinfection.com



Review

Mycobacterium tuberculosis drug-resistance testing: challenges, recent developments and perspectives

T. Schön^{1,2,3}, P. Miotto⁴, C.U. Köser⁵, M. Viveiros^{3,6}, E. Böttger^{3,7}, E. Cambau^{3,8,9,10,*}

WHO general guidelines on treatment of tuberculosis 2000-2010:

“If a history of prior anti-TB drug use suggests that a drug is likely to be ineffective, that drug should not be relied on as one of the four core drugs in the regimen, **even if DST shows the strain to be susceptible.**”

Susceptibility testing for isoniazid, rifampicin, the fluoroquinolones, and the injectable agents is **fairly** reliable. For other agents it is less reliable, and **basing individualized treatments on DST for these agents should be avoided.** The clinical effectiveness or ineffectiveness of a drug cannot be predicted by DST with 100% certainty.

Since a fluoroquinolone and an injectable agent form the backbone of MDR treatment, they should be used even if the patient’s *M. tuberculosis* demonstrates resistance to all available drugs in these two classes.”

-> Not a lot of confidence in AST...

WHO 2014

TABLE 3.3 DST methods and critical concentrations for first- and second-line DST

DRUG GROUP ^a	DRUG	STANDARDIZED DST METHOD AVAILABLE	DST CRITICAL CONCENTRATIONS (µg/ml)			
			Löwenstein- Jensen ^b	Middlebrook 7H10 ^b	Middlebrook 7H11 ^b	MGIT960
Group 1	Isoniazid	Solid, liquid	0.2	0.2	0.2	0.1
First-line oral anti-TB agents	Rifampicin ^c	Solid, liquid	40.0	1.0	1.0	1.0
	Ethambutol ^d	Solid, liquid	2.0	5.0	7.5	5.0
	Pyrazinamide	Liquid	-	-	-	100.0
	Streptomycin ^e	Solid, liquid	4.0	2.0	2.0	1.0
Injectable anti-TB agents	Kanamycin	Solid, liquid	30.0	5.0	6.0	2.5
	Amikacin	Solid, liquid	30.0	4.0	-	1.0
	Capreomycin	Solid, liquid	40.0	4.0	-	2.5
	Ofloxacin ^f	Solid, liquid	4.0	2.0	2.0	2.0
Fluoroquinolones	Levofloxacin	Solid, liquid	-	1.0	-	1.5
	Moxifloxacin ^g	Solid, liquid	-	0.5/2.0	-	0.5/2.0
	Gatifloxacin ^h	Solid	-	1.0	-	-
	Ethionamide	Solid, liquid	40.0	5.0	10.0	5.0
Oral bacteriostatic second-line anti-TB agents	Prothionamide	Solid, liquid	40.0	-	-	2.5
	Cycloserine	Solid	30.0	-	-	-
	<i>Para</i> -aminosalicylic acid	Solid, liquid	1.0	2.0	8.0	4.0

Evaluation of wild-type MIC distributions as a tool for determination of clinical breakpoints for *Mycobacterium tuberculosis*

Thomas Schön^{1*}, Pontus Juréen², Christian G. Giske³, Erja Chryssanthou³, Erik Sturegård⁴,
Jim Werngren², Gunnar Kahlmeter^{5,6}, Sven E. Hoffner² and Kristian A. Ängeby³

JOURNAL OF CLINICAL MICROBIOLOGY, May 2010, p. 1853–1858
0095-1137/10/\$12.00 doi:10.1128/JCM.00240-10
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Vol. 48, No. 5

Wild-Type MIC Distributions for Aminoglycoside and Cyclic Polypeptide Antibiotics Used for Treatment of *Mycobacterium tuberculosis* Infections[∇]

P. Juréen,¹ K. Ängeby,² E. Sturegård,³ E. Chryssanthou,² C. G. Giske,² J. Werngren,¹ M. Nordvall,
A. Johansson,⁴ G. Kahlmeter,^{5,6} S. Hoffner,¹ and T. Schön^{7*}

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doi:10.5588/ijtld.10.0238

Wild-type distributions of seven oral second-line drugs against *Mycobacterium tuberculosis*

T. Schön,* P. Juréen,[†] E. Chryssanthou,[‡] C. G. Giske,[‡] E. Sturegård,[§] G. Kahlmeter,[¶] S. Hoffner,[†]
K. A. Ängeby[‡]

Journal of Antimicrobial Chemotherapy Advance Access published March 23, 2010

Journal of
Antimicrobial
Chemotherapy

J Antimicrob Chemother
doi:10.1093/jac/dkq091

Wild-type MIC distributions of four fluoroquinolones active against *Mycobacterium tuberculosis* in relation to current critical concentrations and available pharmacokinetic and pharmacodynamic data

K. A. Ängeby^{1*}, P. Jureen², C. G. Giske³, E. Chryssanthou¹, E. Sturegård³, M. Nordvall⁴, A. G. Johansson⁴,
J. Werngren², G. Kahlmeter^{5,6}, S. E. Hoffner² and T. Schön⁷

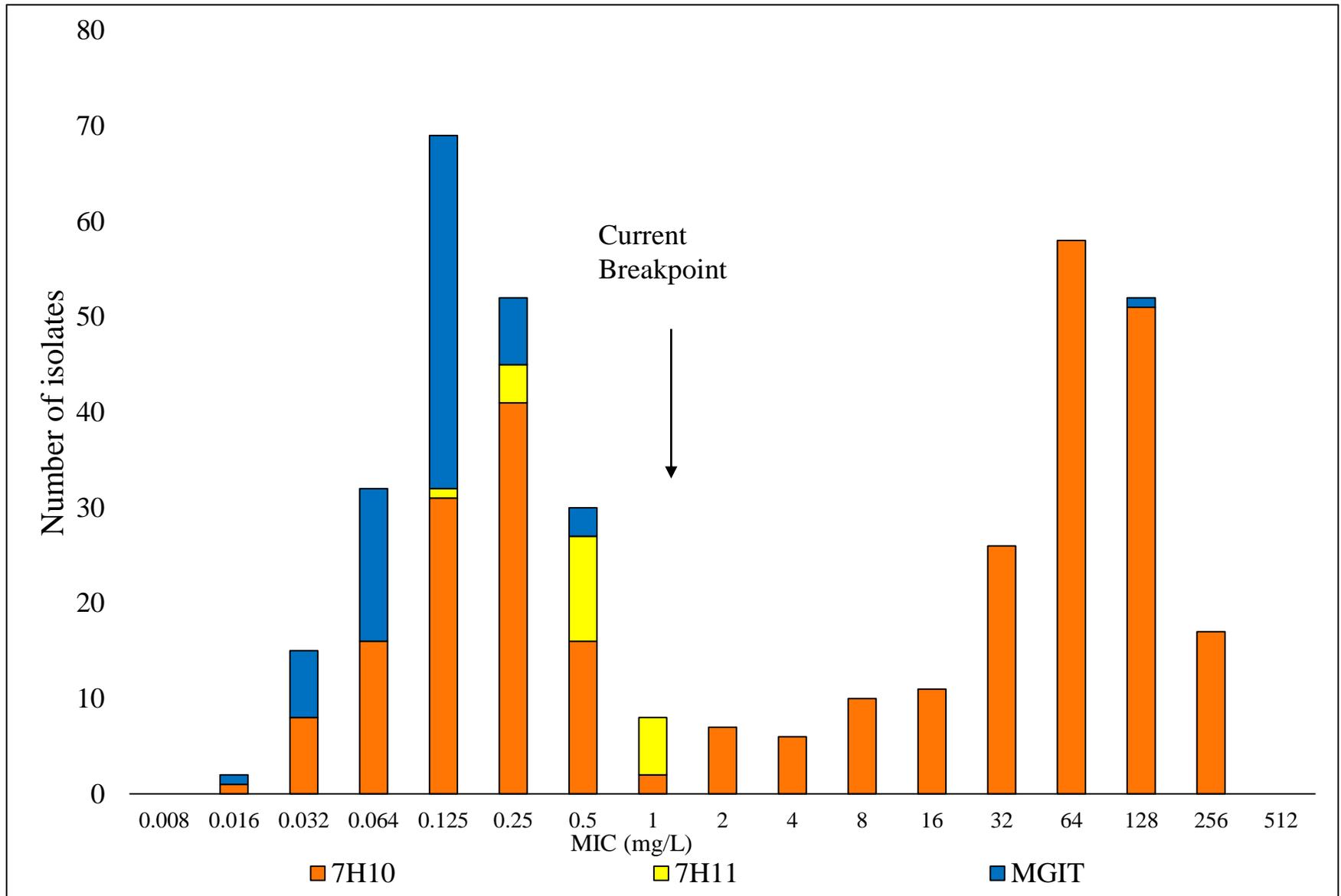
J Antimicrob Chemother 2013; 68: 2074–2077
doi:10.1093/jac/dkt150 Advance Access publication 30 April 2013

Journal of
Antimicrobial
Chemotherapy

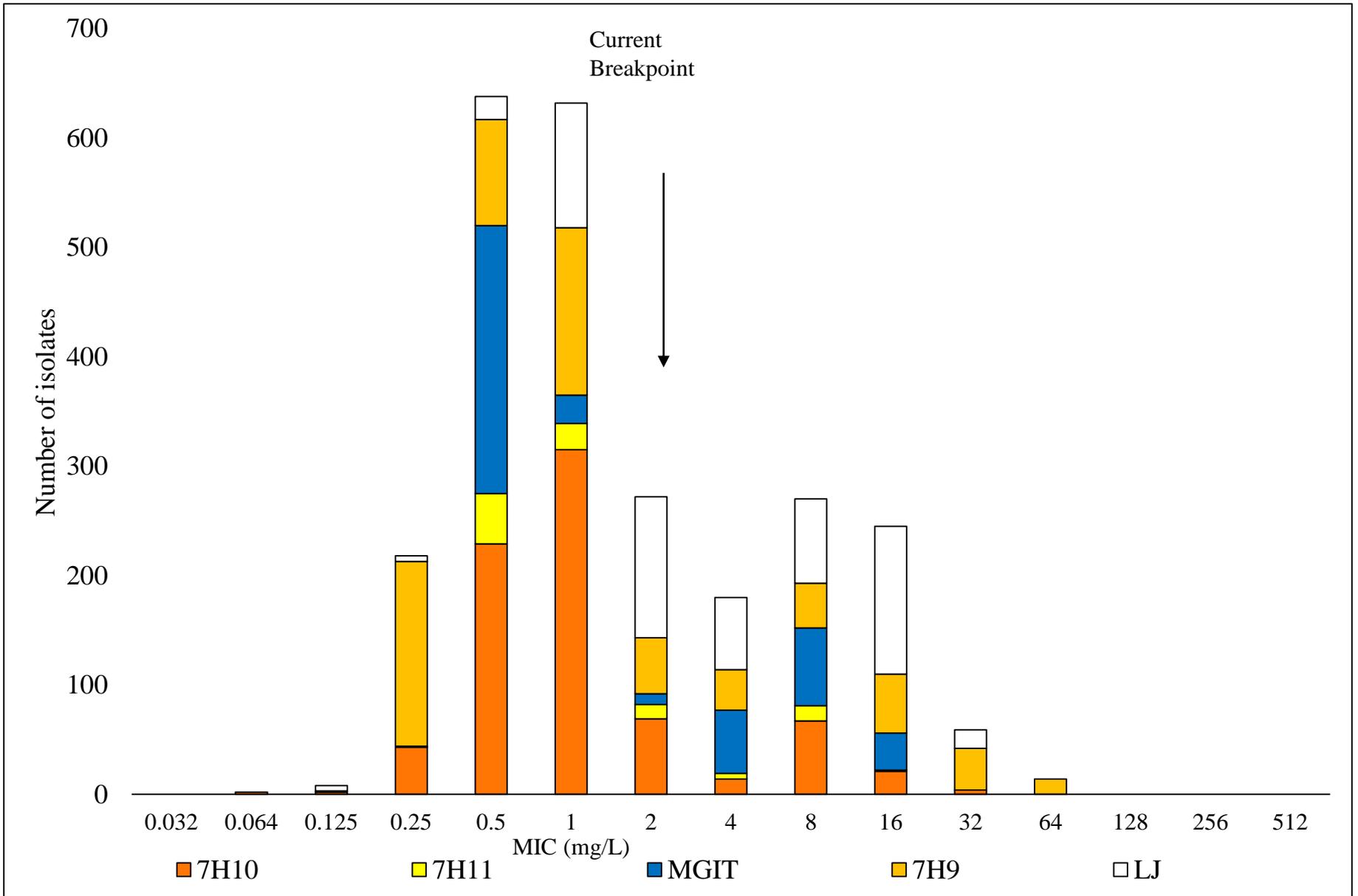
Rifampicin-resistant and rifabutin-susceptible *Mycobacterium tuberculosis* strains: a breakpoint artefact?

T. Schön^{1,2*}, P. Juréen³, E. Chryssanthou⁴, C. G. Giske⁴, G. Kahlmeter^{5,6}, S. Hoffner³ and K. Ängeby⁴

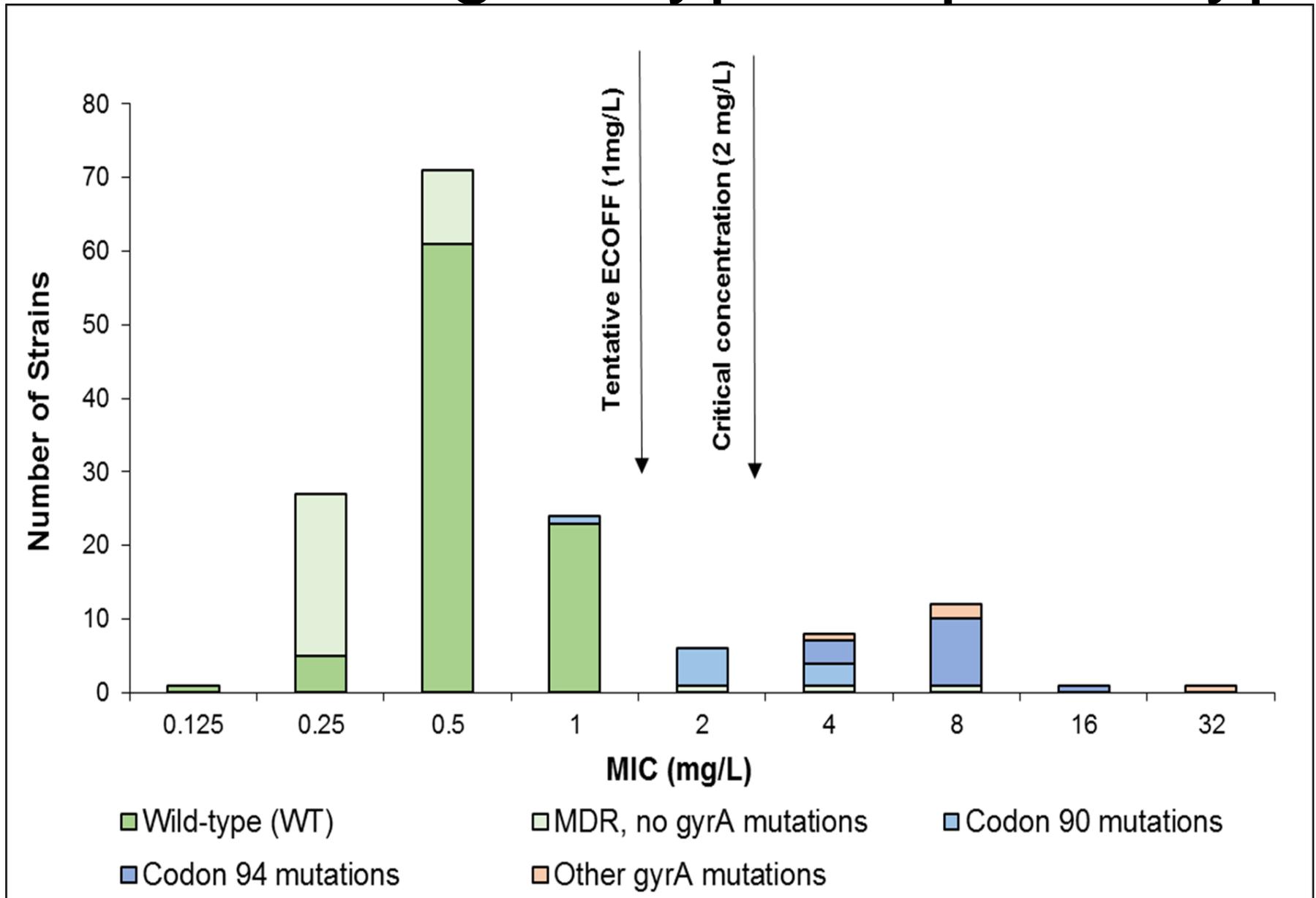
Rifampicin MIC-distribution



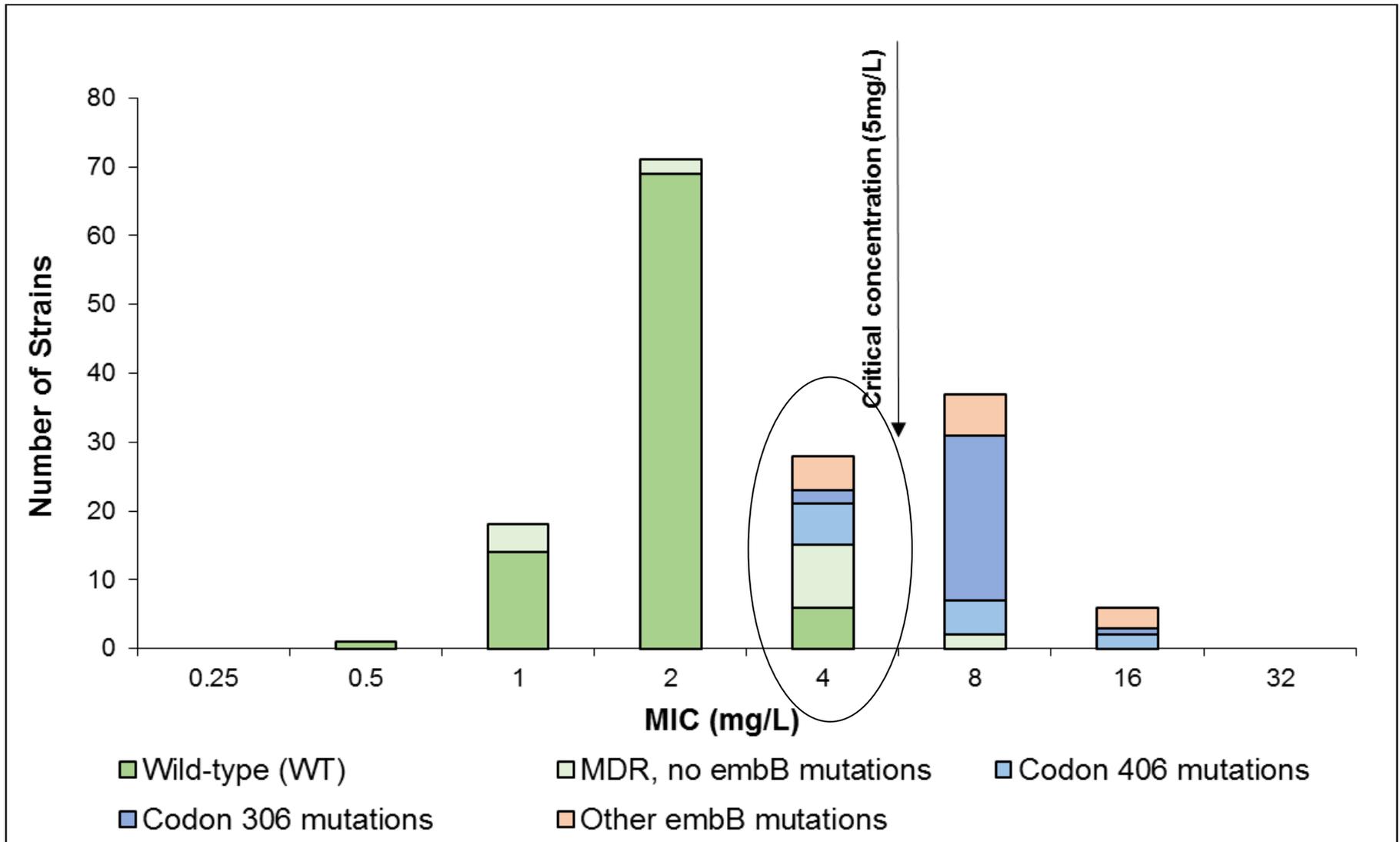
Ofloxacin MIC-distribution



Ofloxacin genotype vs phenotype



Ethambutol – poor reproducibility



Schön T et al JAC 2009
and unpublished observations

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

EUCAST subcommittee on Anti-Mycobacterial Susceptibility Testing (AMST)

- **Initiated at ECCMID Amsterdam, 2016**
- Chair: Emmanuelle Cambau,
- Scientific Secretary: Thomas Schön
- ESGMYC members: Miguel Santin and Miguel Viveiros
- ERL-net-TB members: Daniella Cirillo and Jim Werngren
- EUCAST members: Gerard Lina and Johan Mouton

Strategies and tasks for AMST

- Use the EUCAST strategy/SOPs for setting ECOFFs and clinical breakpoints
 - Epidemiological wild-type cut offs (ECOFFs)
 - Collect available MIC data – separate by method
- Clinical breakpoints and rational documents for new drugs
- Develop a reference method for susceptibility testing of *Mycobacterium tuberculosis*
 - Method protocol developed for Middlebrook 7H10 and 7H9
 - Testing in 7H10 nearly completed for INH, AMI and LEV at 4 laboratories for reference strain *Mycobacterium tuberculosis* H37Rv ATCC 27294
 - Testing initiated in a Middlebrook 7H9 (INH, AMI and LEV at 4 laboratories H37Rv).



Clinical breakpoints - bedaquilin and delamanid

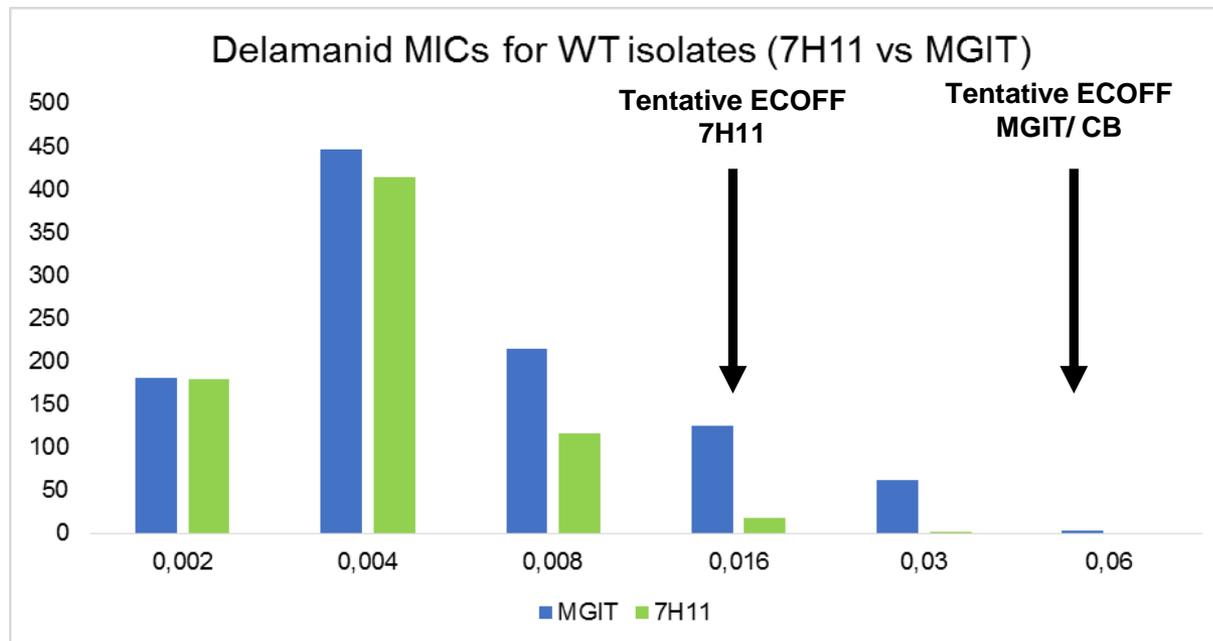
Mycobacterium tuberculosis

EUCAST Clinical Breakpoint Tables v. 8.0, valid from 2018-01-01

Listed breakpoints have been set in parallel with marketing authorisation by EMA. Breakpoints for other agents have not yet been established.

Recommended methods for antimicrobial susceptibility testing of mycobacteria are currently under discussion.

	MIC breakpoint (mg/L)		Notes Numbered notes relate to general comments and/or MIC breakpoints.
	S ≤	R >	
Delamanid	0.06	0.06	1. Breakpoints apply only to tests performed on Middlebrook 7H11/7H10 medium. Comparability of tests performed by other media
Bedaquiline	0.25 ¹	0.25 ¹	has not been established.



WHO TB review – 2018

Technical Report on
**critical concentrations
for drug susceptibility testing
of medicines used in the
treatment of drug-resistant
tuberculosis**



Critical concentration of an anti-tuberculous agent has been adopted and modified from international convention. The critical concentration is defined as the lowest concentration of an anti-TB agent *in vitro* that will inhibit the growth of 99% of phenotypically wild type strains of *M. tuberculosis* complex.

- The **ECOFF** corresponds to the upper end of the pWT distribution (i.e. it typically encompasses 99% of pWT strains).

lower concentration of some agents, the “critical concentration” category is applied. Testing of an additional higher concentration (a clinical breakpoint concentration) may also be recommended for some agents. However, there is no “intermediate” interpretive category, even when testing is performed both at the critical concentration and the clinical breakpoint concentration.

Linezolid MIC in Middlebrook 7H10

Studies	Isolate origin	Unique isolates	Total MICs	Type of isolates	LZD MIC (mg/L)								
					0.06	0.12	0.25	0.5	1	2	4	8	
1) Wang 2007	clinical	420	420	different levels of R	7	28	125	39	204	16	1		
2) Weiss 2015	clinical	1	20	H37Rv		1	14	5					
		130	130	non-MDR		4	121	5					
3) Schön 2011	clinical	18	18	MDR			10	8					
		1	4	H37Rv ATCC 27294			4						
4) Ahmed 2013	clinical	110	110	mostly pan-S		2	91	17					
		1	1	H37Rv ATCC 27294			1						
5) McGee 2009	clinical	43	43	pre-XDR		5	35	3					
		59	59	XDR		6	50	2	1				
6) Koh 2009	clinical	1	1	H37Rv ATCC 27294					1				
		19	19					10	9				
7) Alcalá 2003	clinical	1	1	H37Rv ATCC 25618				1					
		10	10	MDR & XDR			3	7					
8) Yang 2012	clinical	1	1	H37Rv ATCC 27294			1						
		105	105	mostly pan-S		1	4	87	13				
9) Pholwat 2011, 2012 & 2015	clinical	12	12	MDR			5	7					
		1	1	H37Rv ATCC 27294				1					
		15	15	pan-S		1	12	2					
		8	8	INH-R		1	7						
10) van Ingen 2010	clinical	45	45	MDR		5	40						
		16	16	XDR		3	13						
9) Pholwat 2011, 2012 & 2015	clinical	1	1	H37Rv ATCC 27294					1				
		37	37	different levels of R		1	26	10					
10) van Ingen 2010	clinical	1	1	H37Rv ATCC 27294			1						
		28	28	MDR		13	14	1					

Table 1. Critical concentrations and clinical breakpoints for medicines recommended for the treatment of rifampicin-resistant and multidrug-resistant TB.

Drug groups	Drug	LJ	7H10	7H11	MGIT ⁽¹⁾	
A. Fluoroquinolones ⁽²⁾	Levofloxacin (CC) ⁽³⁾	2.0	1.0	–	1.0	
	Moxifloxacin (CC) ⁽³⁾	1.0	0.5	0.5	0.25	
	Moxifloxacin (CB) ⁽⁴⁾	–	2.0	–	1.0	
	Gatifloxacin (CC) ^(3, 5)	0.5	–	–	0.25	
B. Second-line injectable agents	Amikacin	30.0	2.0	–	1.0	
	Capreomycin	40.0	4.0	–	2.5	
	Kanamycin ⁽⁶⁾	30.0	4.0	–	2.5	
	(Streptomycin) ⁽⁷⁾	4.0	2.0	2.0	1.0	
C. Other second-line agents	Ethionamide ⁽⁷⁾	40.0	5.0	10.0	5.0	
	Prothionamide ⁽⁷⁾	40.0	–	–	2.5	
	Cycloserine / terizidone ⁽⁸⁾	–	–	–	–	
	Linezolid	–	1.0	1.0	1.0	
	Clofazimine ⁽⁹⁾	–	–	–	1.0	
D. Add-on agents (not part of the core MDR-TB regimen)	D1	Pyrazinamide ⁽⁷⁾	–	–	–	100.0
		Ethambutol ⁽⁷⁾	2.0	5.0	7.5	5.0
	D2	Bedaquiline ⁽⁹⁾	–	–	0.25	1.0
		Delamanid ⁽⁹⁾	–	–	0.016	0.06
	D3 ⁽¹⁰⁾	p-aminosalicylic acid ⁽⁷⁾	–	–	–	–
		Imipenem-cilastatin ⁽⁷⁾	–	–	–	–
		Meropenem ⁽⁷⁾	–	–	–	–
		Amoxicillin-clavulanate ⁽⁷⁾	–	–	–	–
		(Thioacetazone) ⁽⁷⁾	–	–	–	–

All concentrations are in mg/L and apply to the proportion method with 1% as the critical proportion. Unless otherwise stated, they are critical concentrations (CCs), as opposed to clinical breakpoints (CBs). Changes to the previous version of the table are highlighted in red. (11)

(1) MGIT is proposed as the reference method for performing DST for second-line anti-TB medicines.

(2) Testing of ofloxacin is not recommended as it is no longer used to treat drug resistant-TB and laboratories should transition to testing the specific fluoroquinolones used in treatment regimens. During this transition, testing of ofloxacin at the CCs (i.e. 4.0 mg/L on LJ, 2.0 mg/L on 7H10, 2.0 mg/L on 7H11 and 2.0 mg/L in MGIT) may be performed instead of testing at the CCs for levofloxacin, moxifloxacin and gatifloxacin, but not for the CBs for moxifloxacin.

(3) Levofloxacin, moxifloxacin and gatifloxacin interim CCs for LJ and gatifloxacin CC for MGIT established despite very limited data.

(4) CBs for 7H10 and MGIT apply to high-dose moxifloxacin (i.e. 800 mg daily).

(5) Gatifloxacin CC on 7H10 withdrawn due to limited evidence.

(6) Kanamycin CC on 7H11 withdrawn due to limited evidence.

(7) Drugs not reviewed as part of this report

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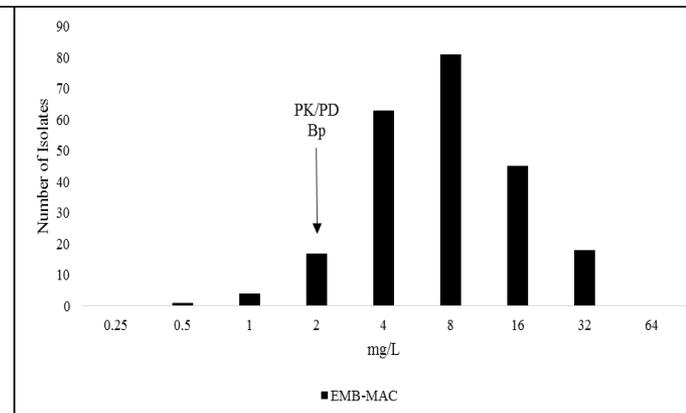
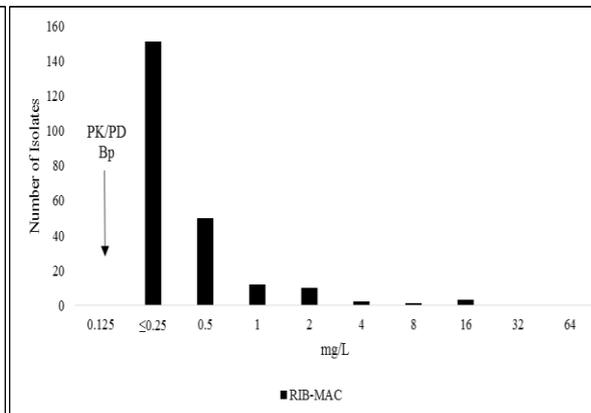
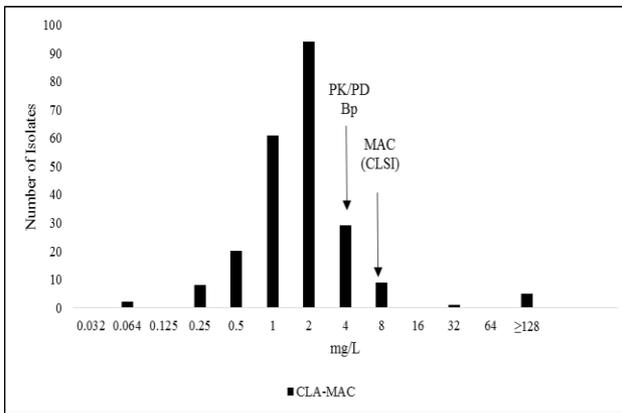
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Indications for AST in NTMs

- MAC (*Mycobacterium avium/intracellulare*)
 - Treatment failure on claritromycin/azitromycin
 - Broth microdilution in Middlebrook 7H9
- *M abscessus* (subspecies!)
 - Planned treatment.
 - Broth microdilution in Middlebrook 7H9
 - HAIN Genotype NTM-DR
 - erm(41): Clarithromycin , rrs: Amikacin
 - *M abscessus* subsp. ***abscessus/massiliense/boletti***

Are we treating NTMs (MAC) with efficient drugs ?

- “Poor correlation between AST and clinical outcome ?”
- **Inefficient drugs used ?**



(mg/L)	0.016	0.032	0.064	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512
Rifampicin	0	0	0	2	1	4	12	30	60	43	77	0	0	0	0	0
Amikacin	0	0	0	0	0	0	3	4	11	52	115	33	9	2	0	0
Moxifloxacin	0	0	0	0	1	10	24	82	85	18	9	0	0	0	0	0
Linezolid	0	0	0	0	0	0	1	6	4	25	75	93	22	3	0	0
TMP/SMX	0	0	0	2	1	9	48	109	41	13	6	0	0	0	0	0

Conclusions

- Too many AST methods for Mycobacteria
 - Urgent need for a reference method !
- MDR/XDR –TB -> Clinical BPs (dosing)
 - NGS/POC – identify drug resistance mutations (5 days vs 4 weeks)
- Clinical breakpoints should be established by ECOFFs, PK/PD and clinical outcome data in breakpoint committees.
- Some of the "old stuff" needs to be critically revised
- The AMST will:
 - work towards defining a reference method for MIC testing for *M. tuberculosis*
 - use MIC-distributions to define ECOFFs and CB by EUCAST criteria

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- The Swedish research council (VR)
- ESGMYC/EUCAST
- Linköping University
- Kalmar County Hospital
- The Swedish Heart and lung Foundation
- Marianne and Marcus Wallenberg Foundation
- The research council of South-East Sweden