World Health Organization

END TB

WHO Policy Update: Interpretation of DST

GLOBAL TB PROGRAMME

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Technical Expert Consultation

FIND conducted systematic review of available minimum inhibitory concentration (MIC) data for phenotypically wild type (pWT) strains, as well as genotypically non-wild type (gNWT) strains, including strains from allelic-exchange experiments for second-line antituberulosis medicines.

The medicines included in the review were the second-line injectable agents (kanamycin, amikacin and capreomycin); clofazimine and bedaquiline; cycloserine and terizidone; linezolid; delamanid; and the fluoroquinolones (ofloxacin, levofloxacin, gatifloxacin and moxifloxacin).

Media reviewed: Löwenstein-Jensen, Middlebrook 7H10/7H11; BACTEC™ Mycobacterial Growth Indicator Tube™ 960 (MGIT).

Phenotypic methods for the diagnosis of DR-TB

Phenotypic, culture methods are based on assessment of the ability of *M. tuberculosis* to grow in culture media (solid or liquid) containing a critical concentration of specific anti-TB agents (which indicates resistance) or, conversely, its inability to grow in the same media (which indicates susceptibility).

The indirect proportion method is the most common method

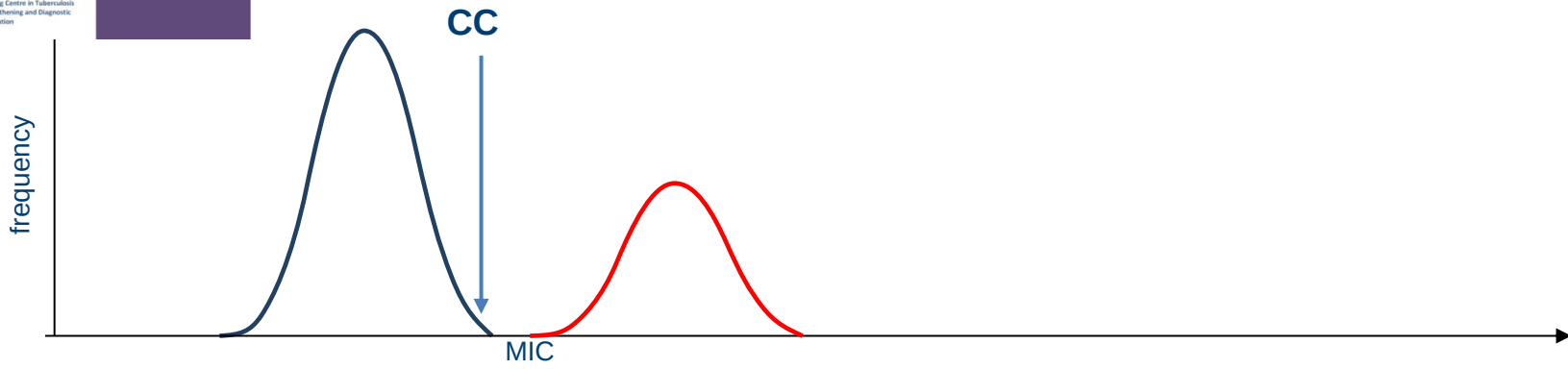
Resistance is defined when at least 1% of growth is observed at the critical concentration of drug in the culture medium.

Commercial liquid culture systems for DST reduce the time to result to as little as 10 days, compared with the 28–42 days needed for DST using solid media

Revised definition: critical concentration

Critical concentration (CC) 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 that will inhibit the growth of at least 95% of wild-type strains of *M. tuberculosis*.

The critical concentration is typically the same or one dilution higher than the epidemiological cut-off value in order not to misclassify phenotypically wild-type strains as phenotypically non wild-type.

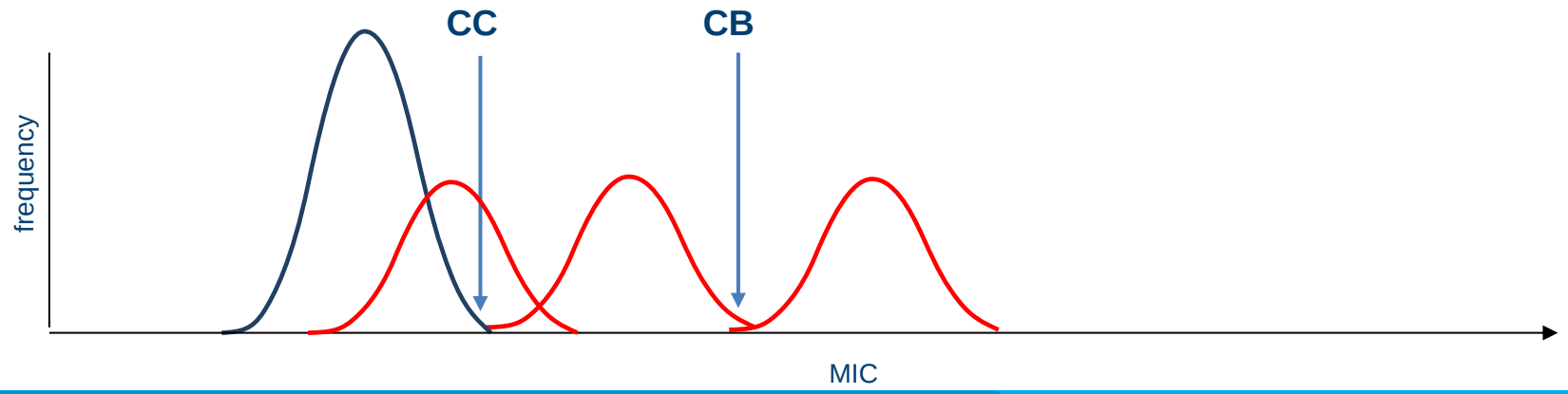


New definition: clinical breakpoint

Clinical breakpoint (CB)- is the concentration or concentrations of an antimicrobial agent which defines an MIC which separates strains which will likely respond to treatment from those which will likely not respond to treatment. This concentration is determined by correlation with available clinical outcome data, MIC distributions, genetic markers, and PK/PD data including drug dose.

A dose increase can be used to overcome resistance observed at lower dosing, up until the maximum tolerated dose, and therefore a higher clinical breakpoint above which the particular drug is not recommended for use.

The clinical breakpoint is commonly different to the critical concentration generally recommended for a given agent.



Phenotypic wild type (pWT) e.g. amikacin MIC distributions on MGIT media

15 studies were identified that reported AMK MIC data for the pWT population with MGIT (Table 23). All studies had MICs for more than 10 pWT isolates. The MIC distributions reaffirmed the CC of 1 mg/L in MGIT.

Studies	Lab	Isolate origin	Unique isolates	Total MICs	Type of isolates	Sequencing results	AMK MIC [mg/L] 0.06	0.12	0.25	0.5	1	1.5	2	2.5	4	8	16	20	32	64	128	256	512	1024
21) Rüsch-Gerdes 2006	15-17	clinical	10	30	H37Rv ATCC 27294 & pan-S					30														
	15-17		21	63	different levels of R					36	9		3	15										
22) Rodrigues 2008	18	clinical	1	1	H37Rv ATCC 27294					1														
	18		10	10	pan-S					10														
	18		20	20	different levels of R					16	2		1	1										
23) Gonzalo 2015	19		1	2	H37Rv				1	1														
	19	clinical	20	40		gWT	1		20	19														
24) Sturegård 2015	3		1	4	H37Rv ATCC 27294					4														
	3	clinical	28	28					8	12	3											1	1	3
25) Heyckendorf	15		1	1	H37Rv ATCC 27294					1														
	15	clinical	9	9	MDR or XDR	gWT			2	7														
26) Tessema 2017	15	clinical	40	40		gWT				19	20				1									
27) Zimenkov 2013	20		1	1	H37Rv ATCC 25618						1													
	20	clinical	33	33		gWT			20	10	3													
28) Kambli 2016a & 2016b	18		1	1	H37Rv ATCC 27294				1															
	18	clinical	31	31		gWT			25	5					1									
29) Matt 2012	21	clinical	10	10	pan-S				5		5													
30) Lin 2009	22		1	3	H37Rv ATCC 27294					3														
	23		1	14						14														
	23	clinical	29	29							14		3	12										
31) Zheng 2016	11		1	1	H37Rv ATCC 25618					1														
	11	clinical	207	207	MDR					38	29		6		14	30	42		12	25	11			
32) Sharma 2011	24	clinical	36	36	different levels of R					22	4		1	9										
33) Cambau 2015	1, 3, 7, 15, 21, 24-27	clinical	113	113	MDR						113													
	1, 3, 7, 15, 21, 24-27		3	3		gWT					1				2									
34) Sirgel 2012	28		1	1	H37Rv ATCC 27294						1													
	28	clinical	15	15	MDR, XDR	gWT					15													
35) Springer 2009	21	clinical	11	11		gWT					11													

The green line denotes the current WHO and CLSI CC for AMK DST by MGIT (1 mg/L). Notable limitations: Only studies 23, 30-32 and 34 have data from unique laboratories.

Systematic review performed by Claudio Köser

Phenotypic wild type (pWT) moxifloxacin MIC distributions on MGIT media

Studies	Lab	Isolate origin	Unique isolates	Total MICs	Type of isolates	Genotypic results	0.06	0.12	0.18	0.25	0.5	0.75	1	1.5	2	2.5	3	4	5	7.5	8
23) Isaeva 2013,	17		1	1	H37Rv ATCC 25618			**1**													
Nosova 2013 &	17	clinical	11	11	different levels of R		1	**9**		1											
Zimenkov 2013	17		23	23		gWT	1	**18**		4											
	18		20	20	H37Rv ATCC 27294 & pan-S		5	**11**		3	1										
24) Piersimoni 2007	18	clinical	10	10			2	**5**		2			1								
	18		1	1		gWT									1						
25) Kambli 2015	19		1	1	H37Rv ATCC 27294		1														
	19	clinical	30	30		gWT	26	2		2											
26) Heyckendorf	20		1	1	H37Rv ATCC 27294					**1**											
	20	clinical	16	16	MDR or XDR	gWT	9	7													
27) Tessema 2017	20	clinical	41	41		gWT	8	**32**		1											
28) Rigouts	2		1	5	H37Rv ATCC 27294			4	1												
	2	clinical	9	9		gWT		9													
29) Sirgel 2012	9		1	6	H37Rv ATCC 27294			6													
	9	clinical	125	125	different levels of R	gWT		119		5					1						
30) van Ingen 2010	8		1	1	H37Rv ATCC 27294			1													
	8	clinical	28	28	MDR			20		1			1	6							
31) Krüüner 2006	21		132	132	MDR			97			5		16	14							
34) Ismail	6	clinical	57	57	different levels of R			26		12	2		2		12			2			1
	6		3	3		gWT		2					1								
33) Lin	22	clinical	73	73		gWT		44		22	1	1	1	3	1						
	22		218	218		gWT				215			2		1						
34) Cambau 2015	3, 8, 20, 23-28	clinical	114	114	MDR					114											
	3, 8, 20, 23-28		3	3		gWT				3											
35) Sharma 2011	29-32	clinical	36	144	different levels of R					101	6		12	25							
36) Kam 2006	33	clinical	108	108	mostly MDR						108										
	33		4	4		gWT							2		2						
	19		1	1	H37Rv ATCC 27294								1								
37) Rodrigues 2008	19	clinical	10	10	pan-S								10								
	19		20	20	different levels of R								19		1						
38) Alvarez 2014	34		1	1	H37Rv					1											
	34	clinical	5	5	MDR or XDR	gWT				1	1		2		1						

The blue line denotes the current CLSI CC for MFX DST by MGIT (0.25 mg/L). The red lines denote the current WHO CCs for MFX DST by MGIT (0.5 and 2 mg/L).

Systematic review performed by Claudio Köser

World Health Organization

Table 1. Medicines recommended for the treatment of rifampicin-resistant and multidrug-resistant TB

Drug groups				LJ proportion	7H10	7H11	MGIT 960
A. Fluoroquinolones [1]		Levofloxacin (CC)	Lfx		1.0	-	1.0
		Moxifloxacin (CC) [2]	Mfx	1.0	0.5	0.5	0.25
		Moxifloxacin (CB) [3]			2.0	-	1.0
		Gatifloxacin [4]	Gfx	0.5	-	-	0.25
B. Second-line injectable agents		Amikacin	Am	30	2.0	-	1.0
		Capreomycin	Cm	40	4.0	-	2.5
		Kanamycin	Km	30	4.0	-	2.5
		(Streptomycin)	(S)	4.0	2.0	2.0	1.0
C. Other second-line agents		Ethionamide	Eto	40	5	10	5
		Prothionamide	Pto	40	-	-	2.5
		Cycloserine / Terizidone [5]	Cs / Trd	-	-	-	-
		Linezolid	Lzd	-	1.0	1.0	1.0
		Clofazimine	Cfz	-	-	-	0.5
D. Add-on agents (not part of the core MDR-TB regimen)	D1	Pyrazinamide	Z	-	-	-	100
		Ethambutol	E	2.0	5.0	7.5	5.0
	D2	Bedaquiline [6]	Bdq	-	-	0.25	1.0
		Delamanid [7]	Dlm	-	-	0.016	0.06
	D3	*p*-aminosalicylic acid	PAS	-	-	-	-
		Imipenem-cilastatin	Ipm	-	-	-	-
		Meropenem	Mpm	-	-	-	-
		Amoxicillin-clavulanate	Amx-Clv				
		(Thioacetazone)	(T)				

CC Critical concentration; CB Clinical breakpoint

Molecular methods for the diagnosis of DR-TB

Molecular (genotypic) methods detect specific DNA mutations in the genome of the *M. tuberculosis*, which are associated with resistance to specific anti-TB drugs.

Molecular methods have considerable advantages for programmatic management of drug-resistant TB, in particular with regard to their speed, the standardization of testing, their potentially high throughput and the reduced requirements for laboratory biosafety.

Molecular tests for detecting drug resistance to rifampicin alone or in combination with isoniazid have been recommended for use by WHO since 2008

Molecular methods for the diagnosis of DR-TB - limitations

There remains imperfect correlation between phenotypic and genotypic methods.

Molecular methods had high specificity but lower sensitivity which varies for different drugs

Rifampicin – *rpo*B 95% sensitivity, 99% specificity

Isoniazid – *inh*A and *kat*G ~90% sensitivity, 99% specificity

Fluoroquinolones – *gyr* A and *gyr*B ~86% sensitivity, 99% specificity

Secondline injectable agents –*rrs* and *eis* ~86% sensitivity, 99% specificity

The predictive values of imperfect tests depend on the pre-test probability of resistance

The ReSeqTB Solution: A Standardized System for Grading Mutations

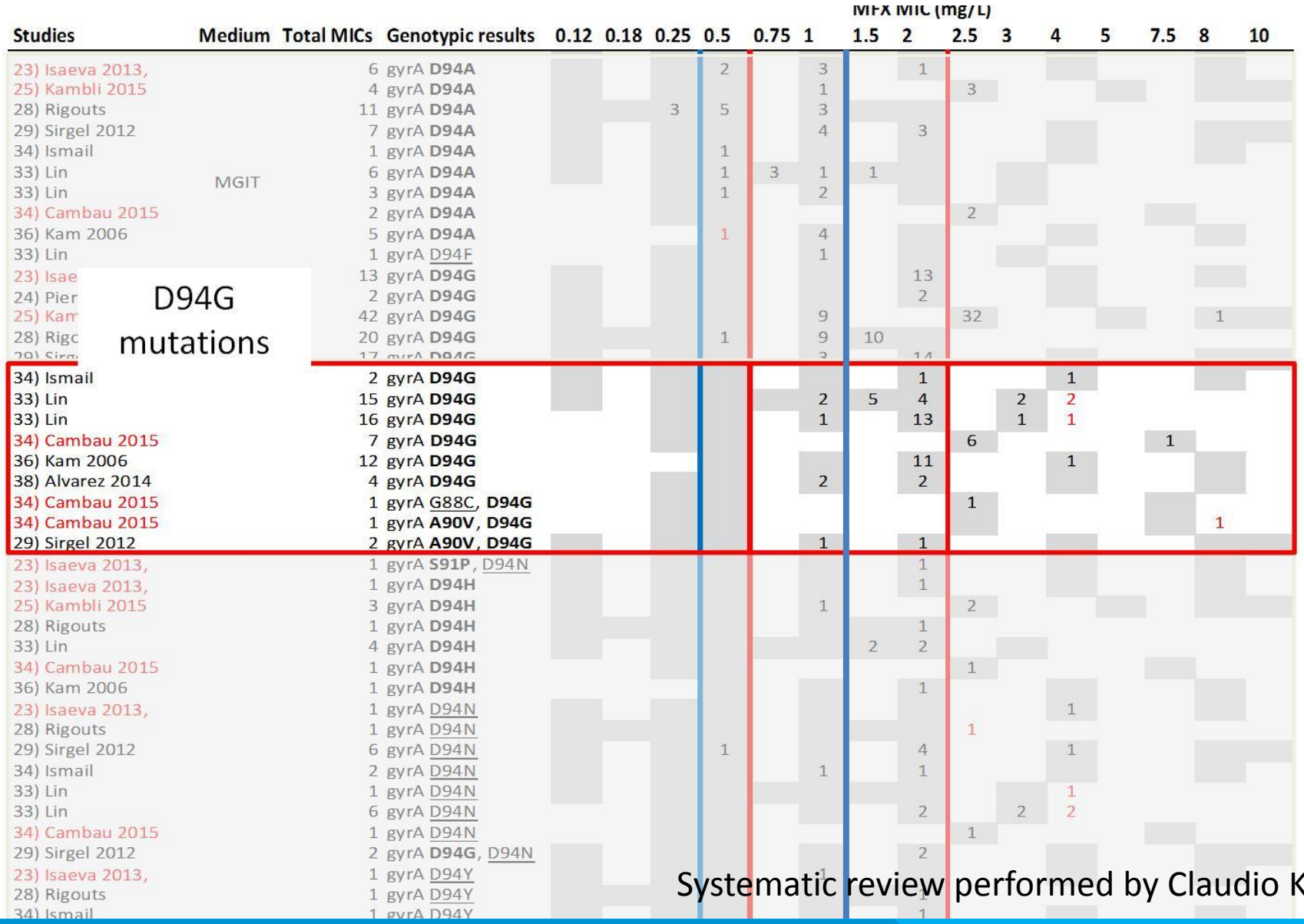
Observed frequency of a mutation found in phenotypically resistant and susceptible strains

- Literature data were used to calculate the frequency of each mutation
- Likelihood ratio (LR) and odd ratio (OR) were used; p-values and 95% confidence intervals associated with LR and OR have been also considered
- Thresholds used most commonly in evidence-based medicine have been adapted to grade *M. tuberculosis* mutations:

LR+ ∨ OR *p*- adj value		Interpretation	Symbol
<0.05	**> 10**	**High Confidence for association with resistance** – strong association of the mutation with phenotypic drug resistanc; sufficient evidence that the mutation confers or is strongly associated with drug resistance	
<0.05	**5 < ... ≤ 10**	**Moderate Confidence for association with resistance** – moderate association of the mutation with phenotypic drug resistanc; additional data desirable for improved evidence that the mutation confers or is strongly associated with drug resistance	
<0.05	**1 < ... ≤ 5**	**Low Confidence for association with resistance** – weak association of the mutation with phenotypic drug resistanc; inconclusive evidence that the mutation confers or is strongly associated with drug resistance. Substantial additional data required	
<0.05	**< 1**	**No association with resistance** – No evidence of association between the mutation and drug resistance	
≥0.05	**-**	**Indeterminate** – no statistically significant threshold reache; additional data required	Indeter

Slide courtesy of Paolo Miotto

NWT MFX MIC distributions of gyrA clinical mutants by MGIT



Systematic review performed by Claudio Köser

Addressing current knowledge gaps

Drug	Gene	Mutation	Confidence value	
			Liquid DST	Solid DST
CM	*rrs*	a1401g	🔴	🟢
H	*inhA*	c-15t	🔴	🟡
KM	*eis*	g-10a	🔴	🟢
R	*rpoB*	L533P	indeter	🟡
R	*rpoB*	D516Y	indeter	🟡
MFX	*gyrA*	A90V	⚫	🟢
R	*rpoB*	L511P	⚫	🔴

Cases testing differently on different media

MIC close to liquid testing critical concetration

Cases missed by liquid phenotypic testing

Slide courtesy of Paolo Miotto

Addressing current knowledge gaps

1. Correlation of phenotypic DST critical concentrations with molecular methods – revised critical concentrations are under review
2. Incomplete cross resistance within the classes of key drugs such as the FQs – mechanisms better understood
3. Technical guide on the use of DNA sequencing as a surveillance tool and the potential use and limitations as a diagnostic tool – under development to determine mutations associated with elevated MICs for certain drugs
4. PK/PD data challenging our dogma around suitable dosing for key drugs especially for moxifloxacin, rifampicin and pyrazinamide

Compendium of WHO guidelines and associated standards:

ensuring optimum delivery of the cascade of care for patients with tuberculosis

THE COMPENDIUM

POLICY
SCREENING
EARLIER DETECTION
DIAGNOSIS
END TB STRATEGY
TREATMENT
DRUG-RESISTANCE
MONITORING & EVALUATION
XDR-TB
COMMUNITY ENGAGEMENT
TB/HIV
MDR-TB
LTBI
TB IN CHILDREN
COMORBID CONDITIONS
PALLIATIVE CARE
PATIENT CARE AND SUPPORT
COMMUNITY
DIGITAL HEALTH
ETHICS
INFECTION CONTROL

THE END TB STRATEGY

World Health Organization

THANK YOU

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