CROSS-RESISTANCE BETWEEN FQ AND DRUG SUSCEPTIBILITY TESTING FOR BDQ AND DLM

MDRTB Symposium
Tbilisi, Georgia
22nd March 2016

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Definitions

Mutant

- Organism with genetic mutations known to confer resistance to a specific drug

Wild type

- Organism without genetic mutations known to confer resistance

Minimum inhibitory concentration (MIC)

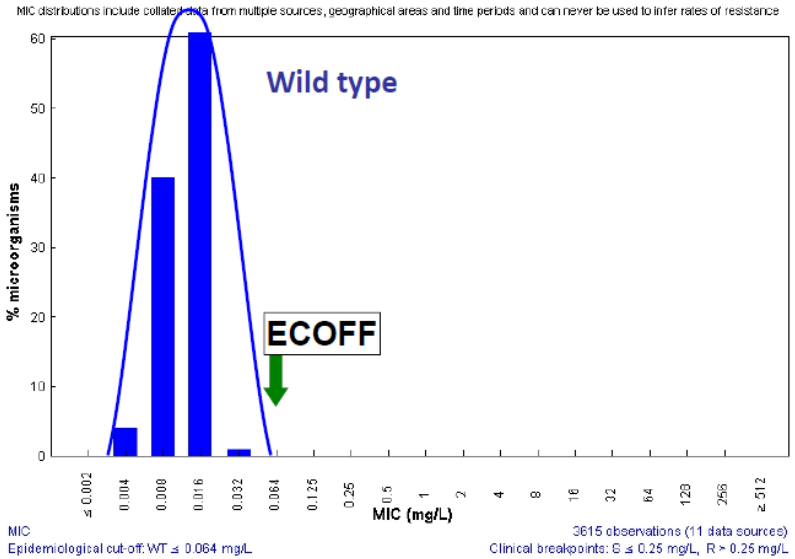
- It refers to a specific strain
- It is the lowest drug concentration inhibiting visible growth of a microorganism
- It is tested as ≥ 5 concentrations with 2-fold dilutions (e.g. 0.125 - 0.25 - 0.5 - 1.0 -2.0 µg/ml)

Critical concentration (or cut-off)

- It refers to a DST method
- It is the lowest drug concentration that inhibits growth of at least 95% of strains never exposed to the drug tested and that simultaneously does not suppress resistant strains

Definitions

- **ECOFF**
  - MIC value identifying the upper limit of the wild type population



MIC value ~ upper limit of the wild type population

- naive population for this drug
- absence of acquired and mutational mechanisms of resistance to the agent

Current recommendations

WHO, 2008

WHO

THE STOP TB DEPARTMENT

Table 2. Current status of DST methodology and critical concentrations for second-line DST

Drug group[a]	Drug	DST category	DST method available	DST critical concentrations (µg/ml)				
				Löwenstein-Jensen[b]	Middlebrook 7H10[b]	Middlebrook 7H11[b]	BACTEC460	MGIT960
Group 1 First-line oral anti-TB agents	Isoniazid	I	Solid, liquid	0.2	0.2	0.2	0.1	0.1
	Rifampicin	I	Solid, liquid	40.0	1.0	1.0	2.0	1.0
	Ethambutol	II	Solid, liquid	2.0	5.0	7.5	2.5	5.0
	Pyrazinamide	II	Liquid	-	-	-	100.0	100.0
Group 2 Injectable anti-TB agents	Streptomycin	II	Solid, liquid	4.0	2.0	2.0	2.0	1.0
	Kanamycin	II	Solid, liquid	30.0	5.0	6.0	4.0	-
	Amikacin	II	Liquid	-			1.0	1.0
	Capreomycin	II	Solid, liquid	40.0	10.0	10.0	1.25	2.5
	Viomycin	V	None	-	-	-	-	-
Group 3 Fluoroquinolones	Ciprofloxacin[a]	III	Solid, liquid	2.0	2.0	2.0	2.0	1.0
	Ofloxacin	III	Solid, liquid	2.0	2.0	2.0	2.0	2.0
	Levofloxacin	IV	Solid, liquid	-	2.0	-	-	2.0
	Moxifloxacin	IV	Liquid	-	-	-	0.5	0.25
	Gatifloxacin	IV	Solid	-	1.0	-	-	-

Critical Concentrations revised in 2012

Drug group[a]	Drug	DST method available	DST critical concentrations (µg/ml)			
			Löwenstein-Jensen[b]	Middlebrook 7H10[b]	Middlebrook 7H11[b]	MGIT960
Group 1 **First-line oral anti-TB agents**	Isoniazid Rifampicin[c] Ethambutol[d] Pyrazinamide	Solid, liquid Solid, liquid Solid, liquid Liquid	0.2 40.0 2.0 -	0.2 1.0 5.0 -	0.2 1.0 7.5 -	0.1 1.0 5.0 100.0
Group 2 **Injectable anti-TB agents**	Streptomycin[e] Kanamycin Amikacin Capreomycin	Solid, liquid Solid, liquid Solid, liquid Solid, liquid	4.0 30.0 30.0 40.0	2.0 5.0 4.0 4.0	2.0 6.0 - -	1.0 2.5 1.0 2.5
Group 3 **Fluoroquinolones**	Ofloxacin[f] Levofloxacin Moxifloxacin[g] Gatifloxacin[h]	Solid, liquid Solid, liquid Solid,liquid Solid	4.0 - - -	2.0 1.0 0.5/2.0 1.0	2.0 - - -	2.0 1.5 0.5/2.0 -
Group 4[i] **Oral bacteriostatic second-line anti-TB agents**	Ethionamide Prothionamide Cycloserine P-aminosalicylic acid	Solid, liquid Solid, liquid Solid Solid, liquid	40.0 40.0 30.0 1.0	5.0 - - 2.0	10.0 - - 8.0	5.0 2.5 - 4.0
Group 5[i] **Anti-TB agents with unclear efficacy (not recommended by WHO for routine use in MDR-TB patients)**	**Clofazimine** **Amoxicillin/clavulanate** **Clarithromycin** **Linezolid**	**Liquid** **None** **None** **Liquid**	- - - -	- - - -	- - - -	- - - 1.0

GLOBAL TB PROGRAMME

World Health Organization

Fluoroquinolones cross-resistance

Correlation of OFX vs GAT vs Moxi (Rigouts 2016)

MIC Ofx > Gat

MIC Ofx = Gat

	GAT MIC (mg/L)							
OFX MIC (mg/L)	**≤ 0.25**	**0.5**	**1**	**2**	**4**	**8**	**>8**	**Tot**
≤ 0.5	12	0	0	0	0	0	0	12
1	83	2	0	0	0	0	0	85
2	59	16	0	0	0	0	0	75
4	1	8	1	0	0	0	0	10
8	0	1	12	3	2	0	0	18
>8	0	0	6	4	5	0	13	28
Tot	155	27	19	7	7	0	13	228

OFX cutoff = 2.0-8.0 mg/L

MXF cutoff= 0.5-2.0 mg/L

MIC Ofx > Mxf

MIC Ofx = Mxf

	MXF MIC (mg/L)							
OFX MIC (mg/L)	**≤ 0.25**	**0.5**	**1**	**2**	**4**	**8**	**>8**	**Tot**
≤ 0.5	12	0	0	0	0	0	0	12
1	75	10	0	0	0	0	0	85
2	35	33	7	0	0	0	0	75
4	0	7	3	0	0	0	0	10
8	0	1	5	10	1	1	0	18
>8	0	0	2	7	2	3	14	28
Tot	122	51	17	17	3	4	14	228

MIC Gat > Mxf

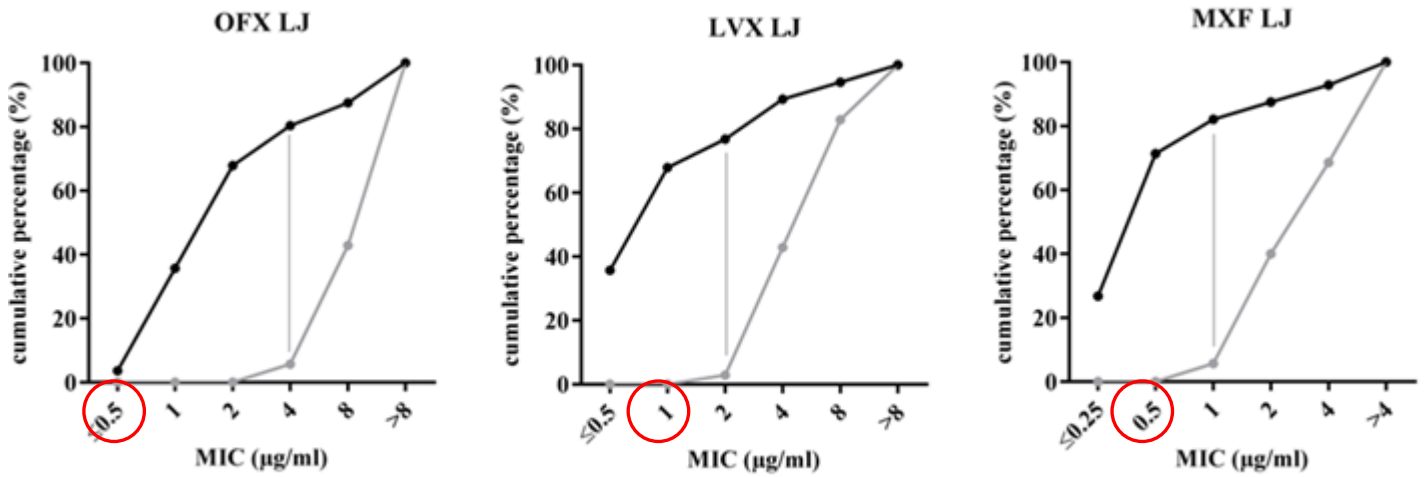
MIC Gat = Mxf

MIC Gat < Mxf

	MXF MIC (mg/L)							
GAT MIC (mg/L)	**≤ 0.25**	**0.5**	**1**	**2**	**4**	**8**	**>8**	**Tot**
≤ 0.25	120	33	2	0	0	0	0	155
0.5	2	17	7	1	0	0	0	12
1	0	1	7	11	0	0	0	85
2	0	0	1	5	1	0	0	75
4	0	0	0	0	2	4	1	10
8	0	0	0	0	0	0	0	18
>8	0	0	0	0	0	0	13	28
Tot	122	51	17	17	3	4	14	228

- ✓ 8 isolates 2.0 mg/L Ofx R and Mfx S
- ✓ 7 isolates 0.5 mg/L Mfx R and Ofx S
- ✓ 1 isolate 8.0 mg/L Ofx S and Mfx R

Correlation between FQs (Coeck et al., 2016)



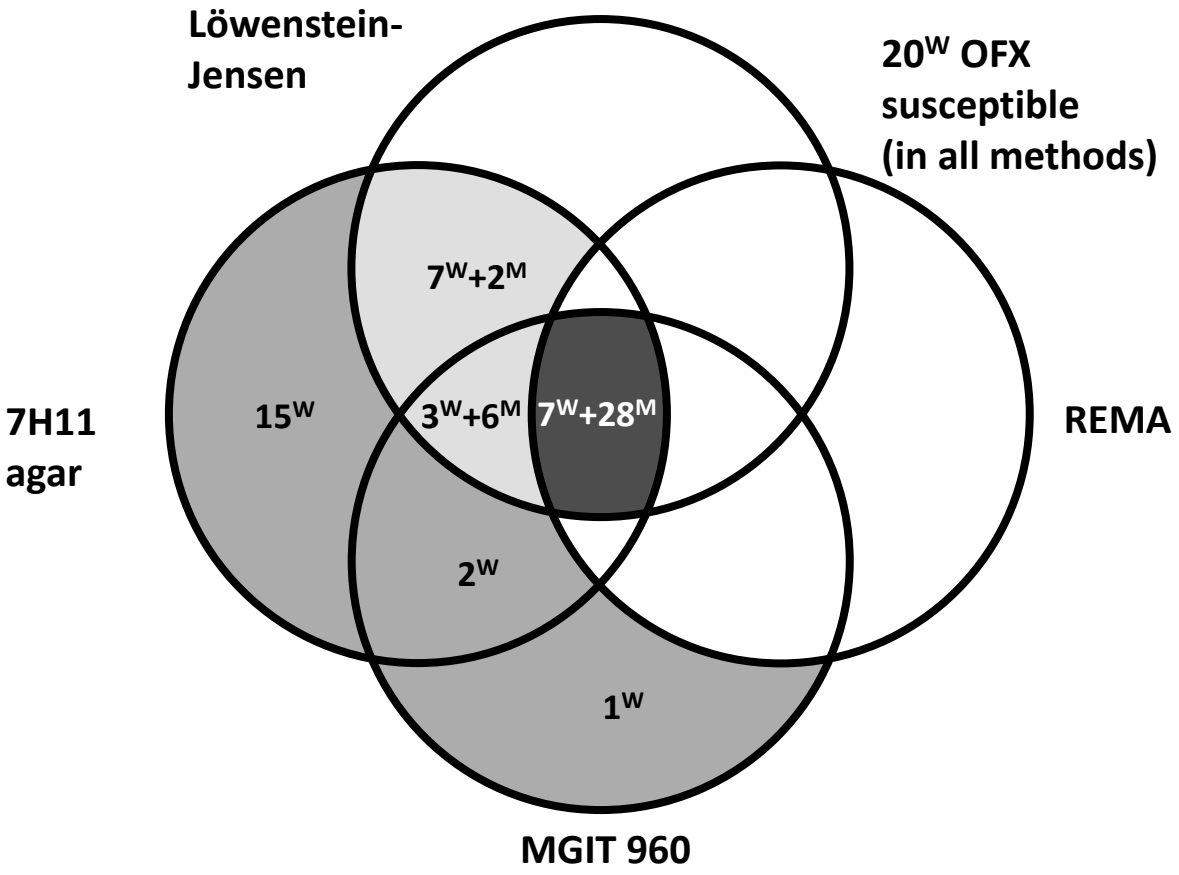
Mutants

WT

MICs on Löwenstein-Jensen

- On LJ of 53 Ofx phenotypical resistant isolates (WT+MUT), 52 (98%) and 50 (94%) were cross resistant to Lfx and Mxf respectively
- All Ofx phenotypical resistant isolates (only MUT) were resistant to Lfx and Mxf

Variability between methods (Coeck 2016)



- For MGIT vs LJ gold standard: Se 83% (increasing to 94% considering only MUT isolates) and Sp 92%
- Overall agreement between methods: 60%

Cross-resistance and clinical correlation

Clinical evidence that the in vitro-activity of newer FQ can overcome resistance to older FQ is sparse:

- On 106 patients Lfx was superior to Ofx 2 mg/ml resistant strains (Yew, 2003)
- On 171 MDR patients: Mox and Lfx showed similar efficacy (Lee et 2011)
- While in-vitro and in-vivo correlation for Ofx and Lfx is known, for moxi this is largely unknow
- Correlation for Gat recently proposed (Rigouts et al, 2016):

GAT MIC (mg/L)	Failure/ relapse	Cure	Total	Percentage cured	OR to fail	95% CI
≤0.25	3	84	87	96.6	1	
0.5	1	17	18	94.4	1.6	0.16–16.8
1	3	14	17	82.4	6.0	1.1–32.8
2	7	9	16	56.3	21.8	4.8–99.3
4	8	7	15	46.7	32.0	6.9–148.5
8	5	0	5	0.0	undef	
>8	3	0	3	0.0	undef	
Total	30	131	161	81.4		

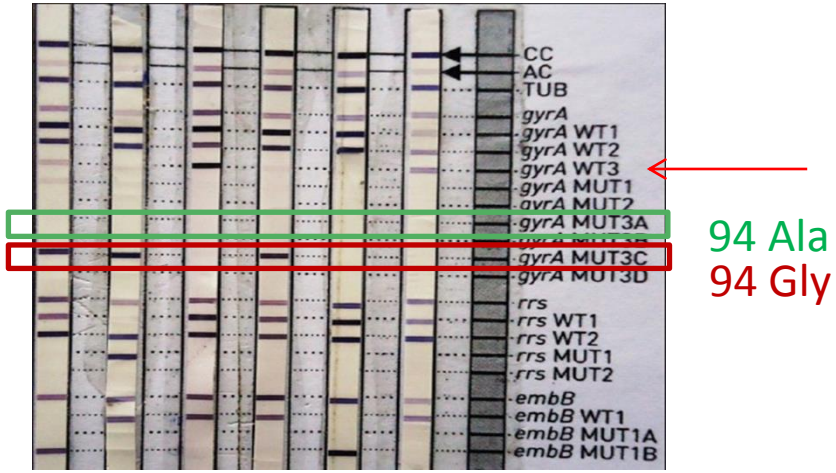
GAT, gatifloxacin; undef, undefined.

The odds of failing significantly increased with the increasing of pre-Tx Gat resistance, with a drop in the proportion of cures of about 50% starting from MIC of 2 mg/L

High dose GAT still cures about 50% patients with GAT resistance

gyr mtations vs MIC

- Most of MUT found in *gyrA* region, correlate to both in-vitro and in-vivo resistance
- Few MUT found in *gyrB*, the majority not conferring phenotypic resistance
- Most common gyrA mutations: 90Val, 94Gly and 94Ala
- All gyrA 94 mutations (except for 94Ala) → high MIC (Gat-Tx cure rate: 30.8%)
- All non-94 gyr mutations + 94Ala → low MIC (Gat-Tx cure rate: 65.6%)
- MDRTB*sl* endorsement under revision (WHO Expert WG Feb 2016)



- Mutations at codon 94 detectable by absence of WT3 probe
- 94Ala linked to MUT 3A, 94Gly to band MUT3C

Detection of FQs resistance

- Only ~50-90% of FQ resistant isolates show MUT
- Also WT isolates found resistant with high MIC
- Unclear role of WT strains with low-level resistance undetected by molecular tests
- Phenotypic tests used as add-on test to detect MUT missed by MDRTB*sl*
- Diagnostic algorithms should include both genotypic and phenotypic tests

DST for Delamanid

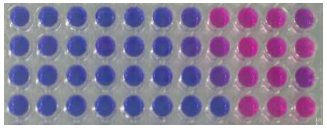
Determination of DLM breakpoint in Milan SRL

- To develop a standardized protocol for rapid Delamanid (DLM) susceptibility testing (DST) using the semi-automated BACTEC™ MGIT™ 960
- To define a breakpoint able to accurately discriminate between susceptibility and resistance of MTB towards DLM.

Validation of the *resazurin microtiter assay* (REMA) and MGIT MIC against APM on a panel of 19 Otsuka pre-characterized strains*

Determination of MIC distribution in REMA and MGIT of clinical isolates never exposed to the drug. Results confirmed by 7H11 agar based protocol (APM)

WGS of study strains to explore genetic polymorphisms in the five genes involved in the F420[Ŧ] mediated activation



Rema plate: based on the reduction of colorimetric indicator (resazurin) caused by the growth of bacteria (color change from blue to pink)

* Strains provided by Otsuka with known level of resistance (control strains)

Ŧ Mutations in genes involved in coenzyme F420 biosynthesis and metabolism **[*fbiA (Rv3261), fbiB (Rv3262), fbiC (Rv1173), fgd1 (Rv0407)*]** has been proposed as possible mechanisms of resistance to DLM (Choi KP *et al*., 2002)

REMA validation: MIC determination of control strains

- Otsuka has independently established a 7H11-based DST method for testing strains and based on MIC performed on a collection of WT and resistant mutant *in vitro* generated strains defined a breakpoint of **0,2 mg/L.**

- Determination of DLM MIC for a panel of 19 reference strains using REMA (from 0,0005 to 32 mg/L), MGIT and 7H11 (from 0,0005 to 16 mg/L):

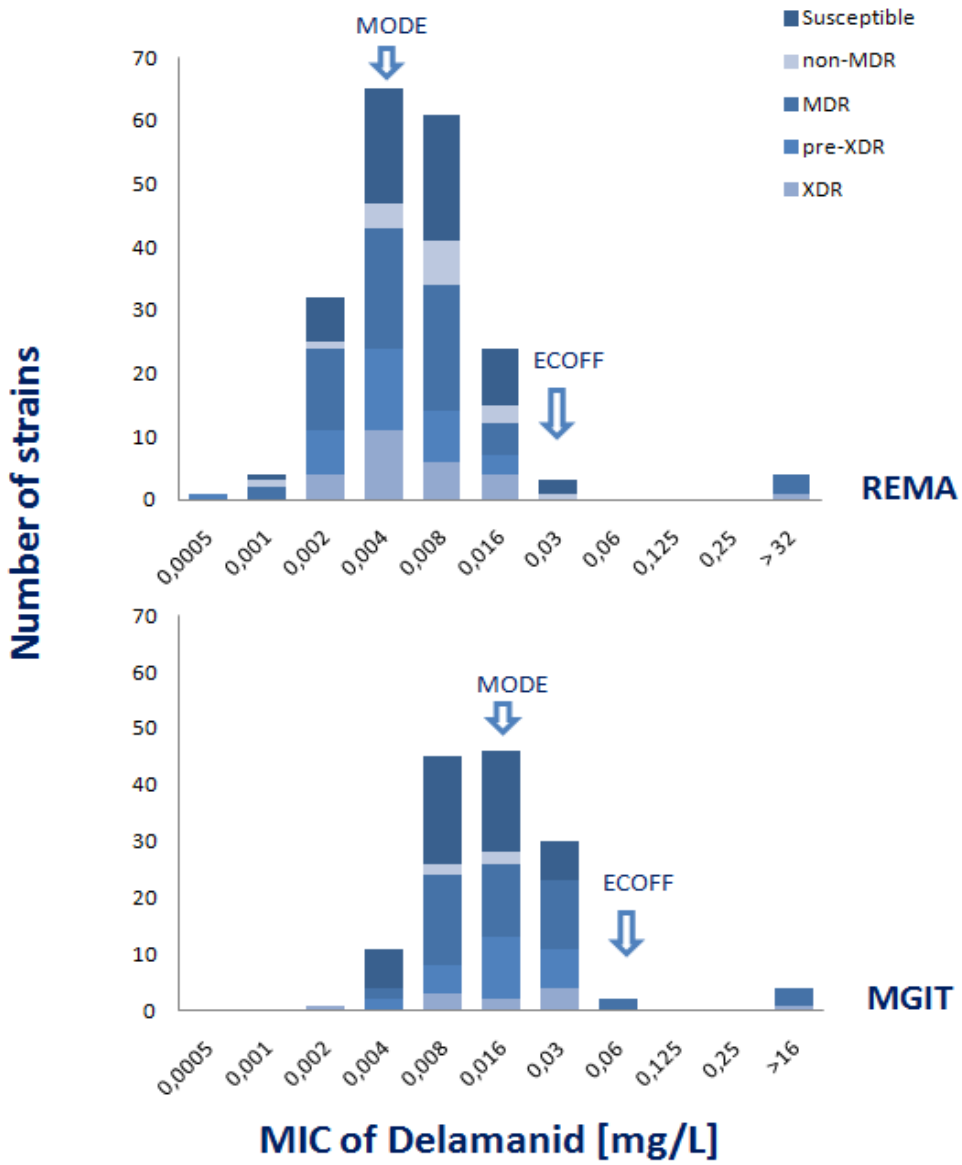
5 resistant strains (MIC > 0.2 mg/L)
14 susceptible strains (MIC < 0.2 mg/L)

100% concordance of all three methods

WGS results for control strains

Code	#strain	MIC	*ddn (Rv3547)*	*fgd (Rv0407)*	*fbiA (Rv3261)*	*fbiB (Rv3262)*	*fbiC (Rv1173)*	Lineage
OTSK	N0268	R	Insertion pos: 3986911	Phe 320 (silent)	wt	wt	wt	Beijing
OTSK	N1002	R	pos 3987149 CA->C DEL	Phe 320 (silent)	wt	wt	wt	Beijing
OTSK	N0185	R	INSERTION + GTCA (pos: 3987023)	wt	wt	wt	Trp678Gly Leu 55 (silent)	LAM
OTSK	N0184	R	Pos 3987023 G->GTCA INS	wt	wt	wt	Trp678Gly Leu 55 (silent)	LAM
OTSK	N0652	R	Leu107Pro	wt	wt	wt	wt	LAM
OTSK	N0339	S	wt	wt	wt	wt	wt	LAM
OTSK	N0085	S	wt	wt	wt	wt	wt	LAM
OTSK	N0001	S	wt	Phe 320 (silent)	wt	wt	wt	Beijing
OTSK	N0082	S	wt	Phe 320 (silent)	wt	wt	wt	Beijing
OTSK	N0299	S	wt	wt	wt	wt	Asp375Asn	LAM
OTSK	N0156	S	wt	Phe 320 (silent)	wt	wt	wt	Beijing
OTSK	N0400	S	wt	Phe 320 (silent)	Val 5 (silent)	wt	wt	EAI "Manila"
OTSK	N0117	S						
OTSK	N0110	S	wt	Phe 320 (silent)	wt	wt	wt	Beijing
OTSK	N0678	S	wt	wt	wt	wt	wt	LAM
OTSK	N0097	S	wt	wt	wt	wt	Try678Gly Leu 55 (silent)	LAM
OTSK	N0667	S	wt	wt	wt	wt	Lys 8 (silent)	Euro-Am Sup
OTSK	N0946	S	wt	wt	wt	wt	wt	Euro-Am Sup
OTSK	N0193	S	wt	Phe 320 (silent)	wt	wt	wt	Beijing

MIC results from clinical isolates



- 1 XDR and 3 MDR strains with high MIC, with resistance confirmed by 7H11 APM

WGS results for clinical isolates

Phenotype	Lineage	*fgd1*	*fbiA*	*fbiB*	*fbiC*	*ddn Rv3547*	*nt change*	MIC
MDR	Beijing	wt	wt	wt	wt	Trp88STOP	TGG->TGA	≥ 32
MDR	Beijing	wt	wt	wt	wt	Trp88STOP	TGG->TGA	≥ 32
MDR	Beijing	wt	wt	wt	wt	Trp88STOP	TGG->TGA	≥ 32
DR	EAI	wt	wt	wt	wt	Arg72Trp	AGG->TGG	0,002
MDR	Ural	wt	wt	wt	wt	Glu83Asp	GAG->GAT	0,001
MDR-AG	EAI	wt	wt	wt	wt	Arg72Trp	AGG->TGG	0,004
MDR	M. africanum WA2	Lys296Glu*	wt	wt	wt	wt	AAG->GAG	0,001
MDR	Harlem	Lys270Ser*	wt	wt	wt	wt	AAG->ATG	0,004
XDR	Beijing	Lys250STOP	wt	wt	wt	wt	AAG->TAG	≥ 32
MDR-FQ	Eur-Am Superlineage	wt	Thr302Met	wt	wt	wt	ACG->ATG	0,001
MDR	Eur-Am Superlineage	wt	Thr302Met	wt	wt	wt	ACG->ATG	0,002
DR	Eur-Am Superlineage	wt	Gln120Arg	Phe220Leu	wt	wt	CAA->CGA;TTC->TTA	0,0016
DR	Eur-Am Superlineage	wt	Gln120Arg	wt	wt	wt	CAA->CGA	0,008
S	Eur-Am Superlineage	wt	Gln120Arg	wt	wt	wt	CAA->CGA	0,004
S	Eur-Am Superlineage	wt	Gln120Arg	wt	wt	wt	CAA->CGA	0,008
MDR	Beijing	wt	wt	Phe220Leu	wt	wt	TTC->TTA	0,004
MDR	Delhi/CAS	wt	wt	Lys448Arg	wt	wt	AAG->AGA	0,008
S	Eur-Am Superlineage	wt	wt	Leu447Arg	wt	wt	CTA->CGA	0,004
S	Eur-Am Superlineage	wt	wt	wt	Thr273Ala	wt	ACT->GCT	0,002
S	Eur-Am Superlineage	wt	wt	wt	Thr273Ala	wt	ACT->GCT	0,004
S	Eur-Am Superlineage	wt	wt	Phe220Leu	Thr273Ala	wt	TTC->TTA	0,008
S	Eur-Am Superlineage	wt	wt	wt	Thr273Ala	wt	ACT->GCT	0,008
S	Beijing	wt	wt	wt	wt	Thr681Ile	ACC->ATC	0,004

Conclusion for DLM

→ DST for DLM can be performed in both MGIT and REMA at 0.125 mg/L (close to ECOFF, according to EUCAST definition)

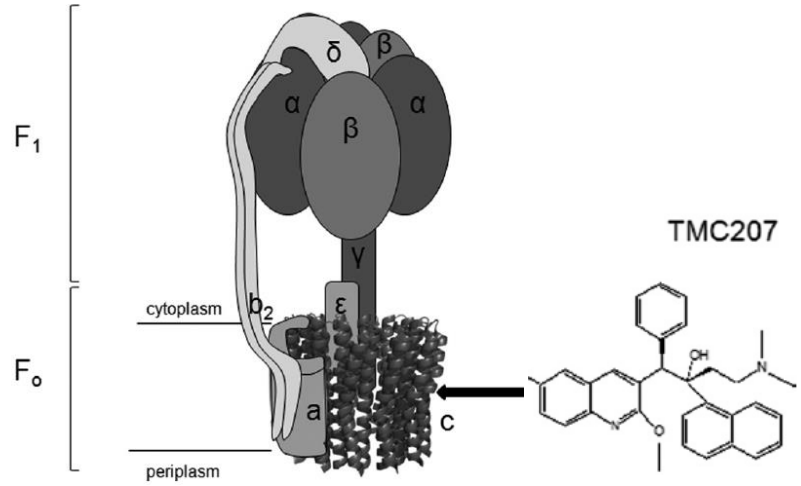
→ Pre-exposure high level resistance was observed on clinical strains

→ Low level resistance was not observed

→ WGS analysis in genes involved in the activation pathways show presence of several mutations non related to an increased MIC

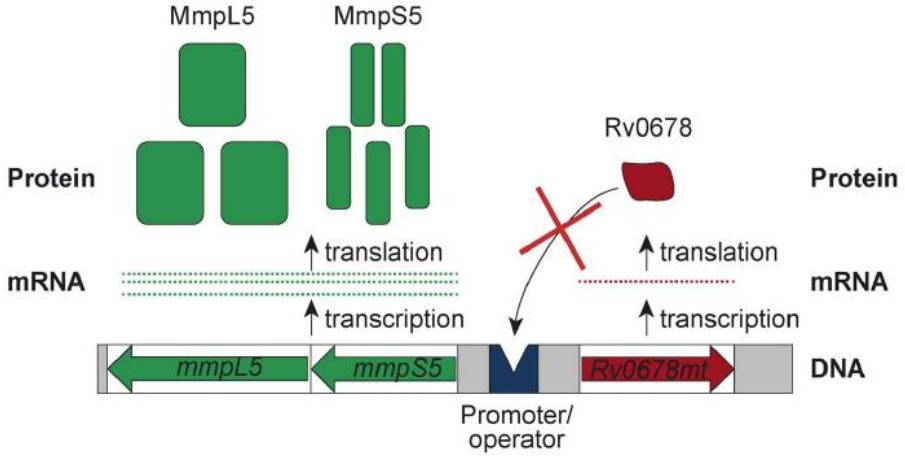
DST for Bedaquiline

TMC207 or Bedaquiline (BDQ)



- **Target:** *atpE* (*Rv1305*) gene that encodes the subunit *c* of ATP synthase

- Off target mutations in *Rv0678* gene cause resistance to BDQ by up-regulation of MmpL5/Mmps5 efflux pump gene expression (Andries K *et al*., *PLoS One* 2014)



- **Cross-resistance between Bedaquiline and Clofazimine (CLF)** (Hartkoorn RC *et al*., *Antimicrobial Agents and Chemotherapy* 2014)

Bedaquiline DST in Clinical trials

Drug susceptibility testing (DST) in the Phase 2 clinical studies (Diacon et al. 2009; Diacon et al. 2012; Diacon et al. 2014; Pym et al. 2013), was performed by:

- 7H11 agar dilution method
- 7H9 broth microdilution method using the resazurin microtitre assay (REMA) (Palomino et al. 2002; Martin et al. 2003)
- the standard QC strain H37Rv is used under the same conditions to ensure that the BDQ MIC of the reference falls within a predefined QC range

QC MIC ranges

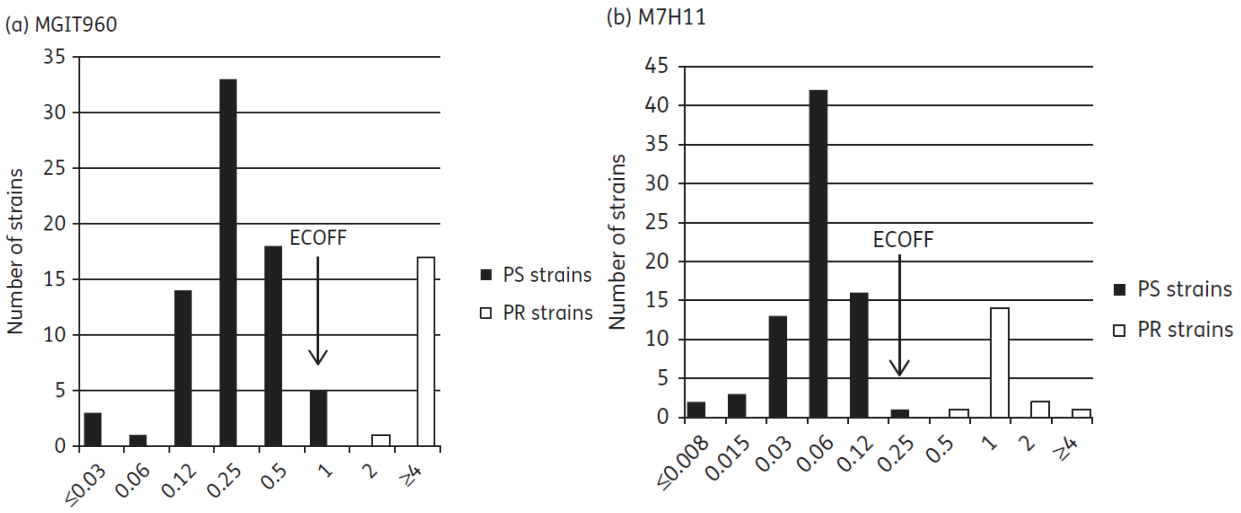
- Provisional BDQ MIC QC ranges against MTB H37Rv were established at Janssen US

- One study carried out in 8 laboratories defined the BDQ MIC QC ranges against the MTB reference strain H37Rv were as 0.015 to 0.12 µg/mL on 7H10 and 7H11 agar and 0.015 to 0.06 µg/mL in 7H9 broth.

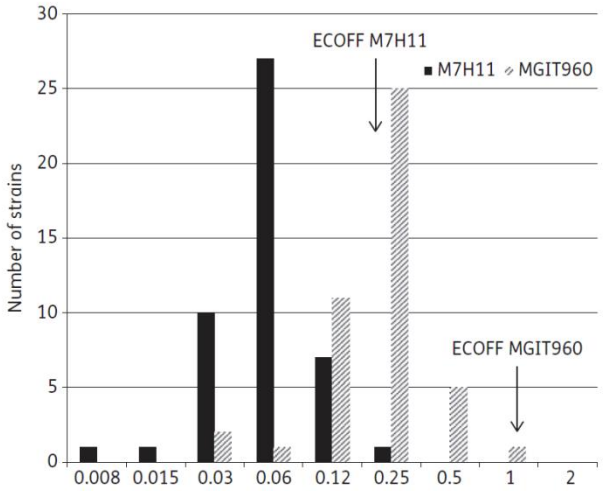
- Validation by independent scientists as an effort to produce standard QC values to guide further tests

Bedaquiline DST on MGIT (Torrea G al, 2015)

Distribution of BDQ MIC in MGIT and 7H11 for PR and PS strains



Distribution of BDQ MIC for WT strains



	M7H11 results		
MGIT results	R	S	Total
R	18	0	18
S	0	74	74
Total	18	74	92

MGIT results obtained using ECOFF 1.0 mg/L breakpoint

7H11 results obtained using ECOFF 0.25 mg/L breakpoint

DST for BDQ: conclusions

Medium	ECOFF (µg/ml)	Breakpoint (µg/ml)	QC range H37Rv (µg/ml)
7H10 agar			0.015 - 0.12
7H11 agar	0.25	0.25	0.015 - 0.12
7H9 broth (REMA)			0.06 – 0.015
MGIT960	1.0	1.0	0.12 – 0.5
LJ	NA	NA	NA

- Cross-resistance between Cfz and BDQ due to common target mutations in *Rv0687* gene have been documented (Andries et al; 2014)
- It is still unknown if prior Cfz treatment affects efficacy of BDQ treatment (Torrea et al; 2015)

Acknowledgments

Slides covering DST for DLM and BDQ were kindly provided Dr Daniela Maria Cirillo, San Raffaele Scientific Institute, Milan, slightly modified for this presentation.

I also thank Dr Leen Rigouts (ITM), for her input on the preparation of the sides on FQ cross-resistance