Residual activity of antituberculous agents on strains with resistant traits

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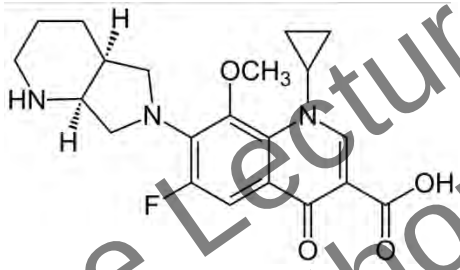
Fluoroquinolones

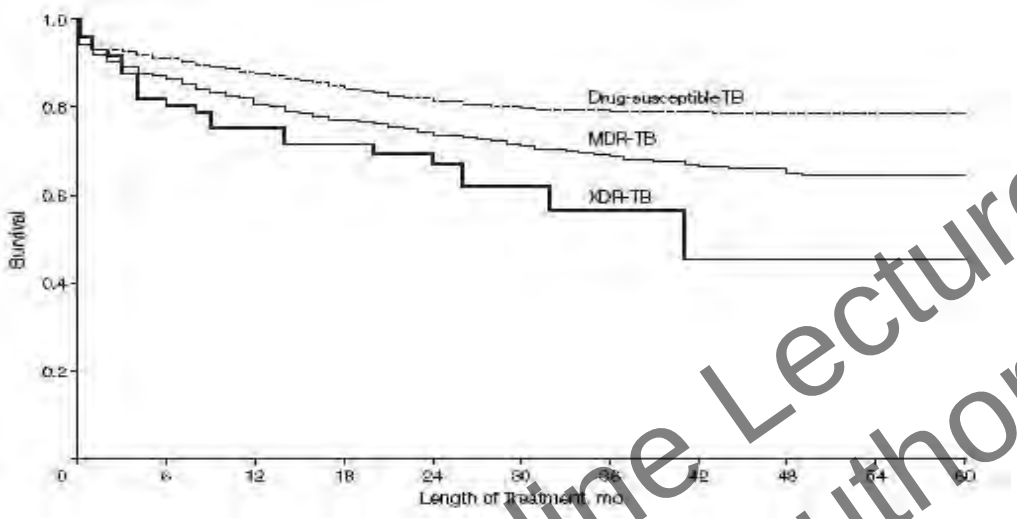
- DNA gyrase inhibitors



moxifloxacin

- Antituberculous activity described 30 years ago
  - Tsukamura, 1985 : first demonstration of activity in human
    - 19 patients, tuberculosis treatment failure
    - Ofloxacin
    - Decrease sputum bacillary load
    - Selection drug resistant mutants
- Tahaoglu, 2001
  - Fluoroquinolones improve MDR TB prognosis

Prognosis of MDR and XDR cases

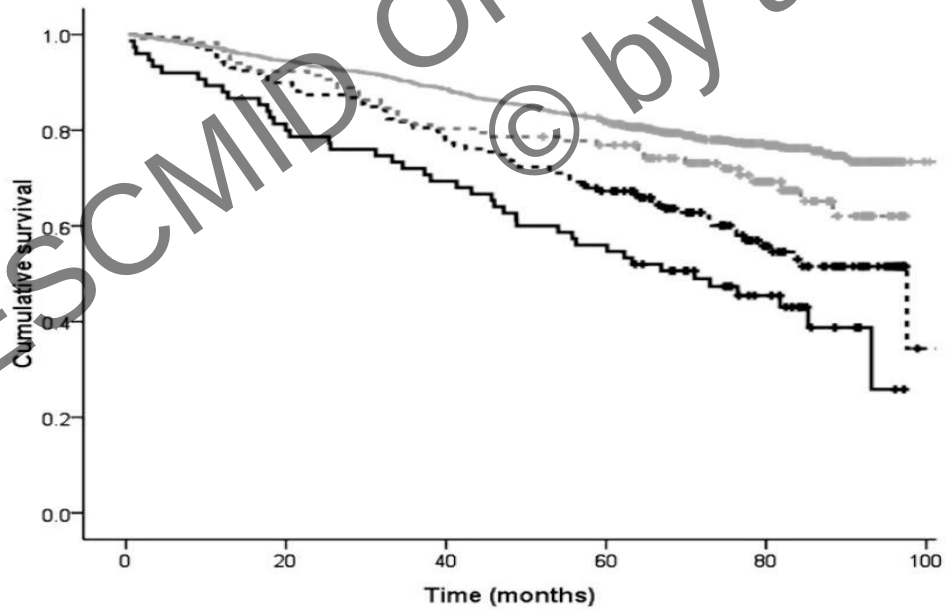


DS TB

MDR TB

XDR TB

Shah et al., JAMA, 2008



MDR TB

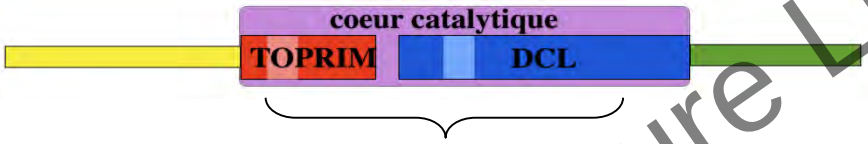
MDR TB + aminoglycosides R

MDR TB + Fluoroquinolones R

XDR TB

Kim et al., AJRCCM, 2010

Fluoroquinolone resistance = OFX MIC > 2 mg/L



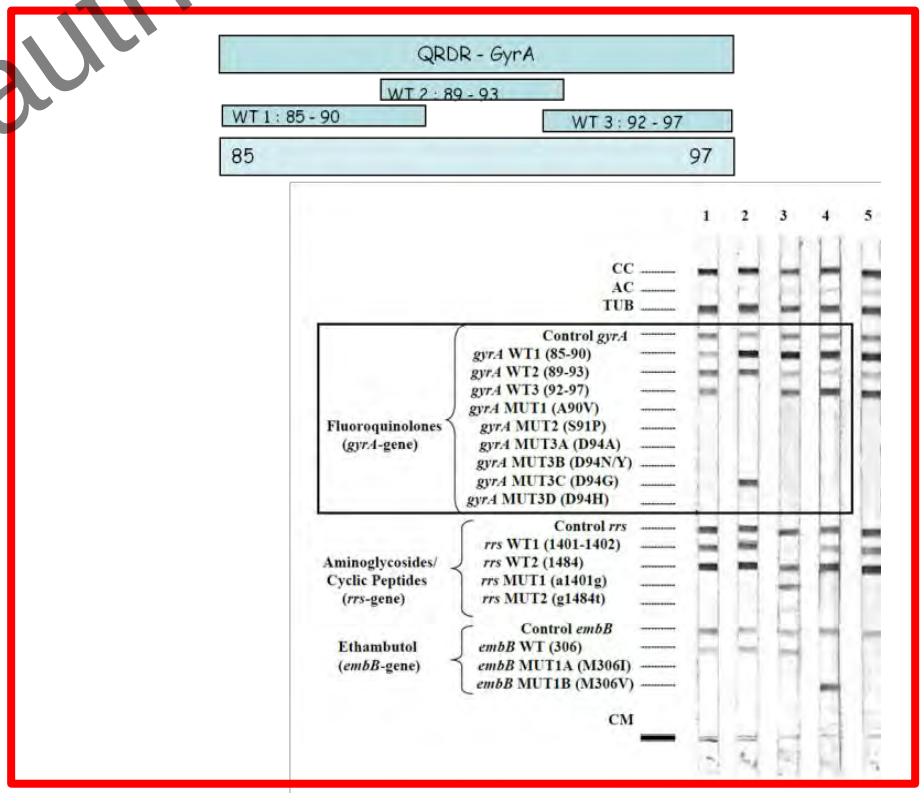
Quinolones binding pocket

QRDR-B (500-538) QRDR-A (73-113)

Can be detected by commercial genotypic test MTBDRs*l*®

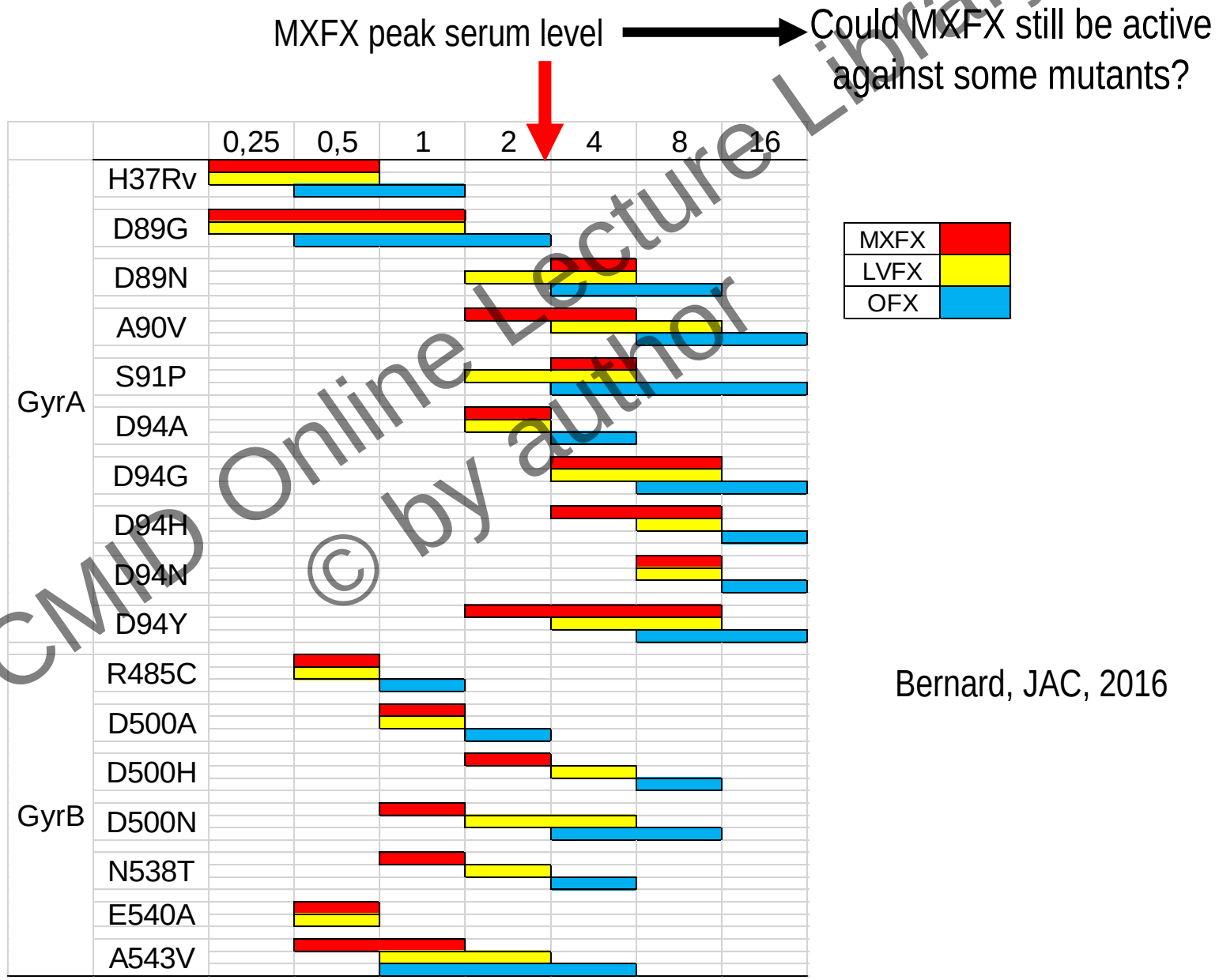
+ aminoglycosides
+ capreomycin
+ ethambutol

+



Brossier IJTLD 2009, Brossier JCM 2010

Various levels of FQ resistance



Bernard, JAC, 2016

Various levels of resistance : 2 sides of a same coin

the bright side : use of drug in case of low-level resistance

M & M

	Mutations		MIC (mg/l)	
Strains	GyrA	GyrB	Moxifloxacin	Ofloxacin
H37Rv	-	-	0,25	0,5
	-	D500N	0,5	4
	A90V		2	>8
	D94G		4	>8

- Isogenic mutants of H37Rv

- Moxifloxacin dosing
  - –100 mg/kg/j or 33 mg/kg/8h
  - –200 mg/kg/j or 66 mg/kg/8h

	H37Rv	GyrA		GyrB	Total
		A90V	D94G	D500N	
J0	10	10	10	10	40
Untreated	10	10	10	10	40
moxifloxacin 100 mg/kg/d	10	10	10	10	40
moxifloxacin 33 mg/kg/8h	10	10	10	10	40
moxifloxacin 200 mg/kg/d	10	10	10	10	40
moxifloxacin 66 mg/kg/8h	10	10	10	10	40
Total	60	60	60	60	240

TABLE 3. Pharmacokinetic data for moxifloxacin in a murine model

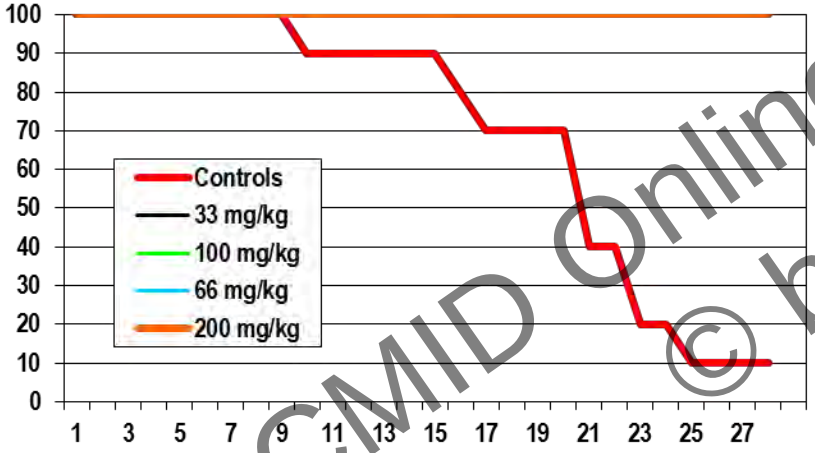
Parameter (unit)	Value for: Mice given moxifloxacin dose (mg/kg×gavages/day) of: 33×3	100×1	66×3	200×1	Humans given 400 mg moxifloxacin/day[a]
T_{max} (min)	22	22	22	30	42
C_{max} (μg/ml)	5	9	7	10	2.5–4.3
$t_{1/2}$ (h)	2.3	1.8	2.1	2.0	9.2–15.6
AUC[b] (μg · h/ml)	24	22	39	27	27–45

[a] This is the standard dose administered to humans (26, 36, 37, 40, 44).
[b] For doses of 33 mg/kg×3/day and 66 mg/kg×3/day, the AUC was obtained by multiplying the AUC measured after one dose by 3.

Poissy, AAC 2010

Results: H37Rv (susceptible strain, MXF MIC = 0,25 mg/l)

% surviving mice depending on dosing



Log10 CFU after 1 month

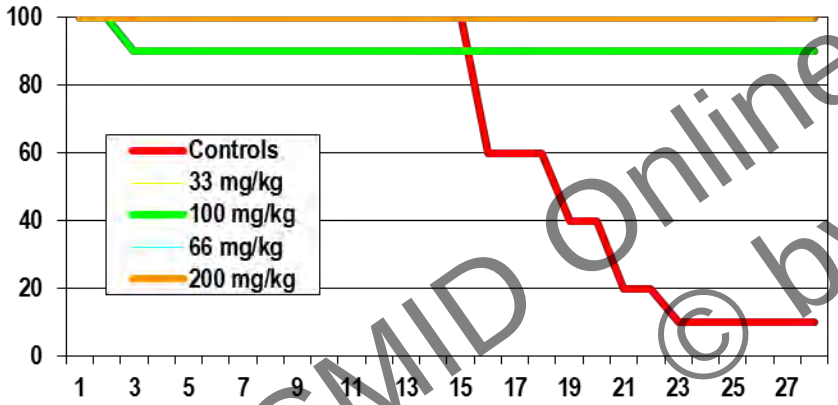
Dosing (mg/kg/j)	33x3	100x1	66x3	200x1
Δ (log10 CFU)	-2,5	-2,5	-3	-3

Poissy, ACC 2010

➲ Prevents mortality
➲ Bactericidal activity

Results: mutant GyrB D500N (MXF MIC = 0,5 mg/l)

% surviving mice depending on dosing



Log10 CFU after 1 month

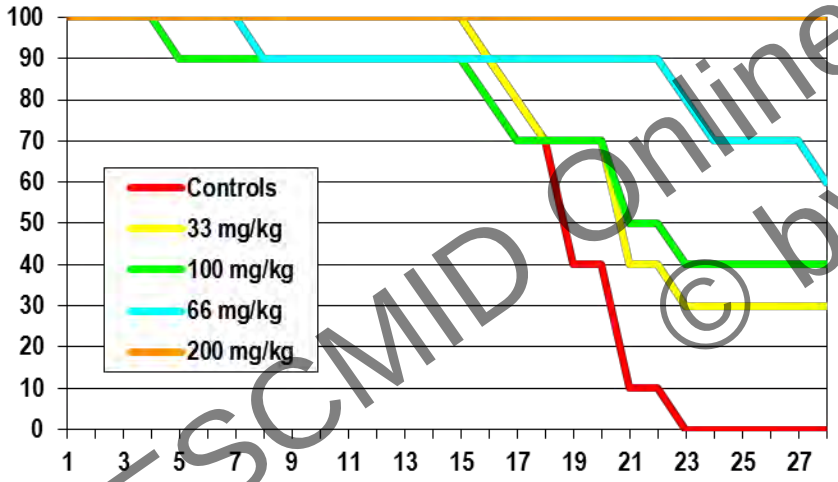
Dosing (mg/kg/j)	33x3	100x1	66x3	200x1
Δ (log10 UFC)	-0,5	-1	-1,8	-1,8

Poissy, AAC 2010

➲Prevents mortality
➲ Bactericidal activity at high dosing

Results: mutant GyrA A90V (MXF MIC = 2 mg/l)

% surviving mice depending on dosing



Log10 CFU after 1 month

Dosing (mg/kg/j)	33x3	100x1	66x3	200x1
Δ(log10 UFC)	+0,5	+1	+1,8	+1,8

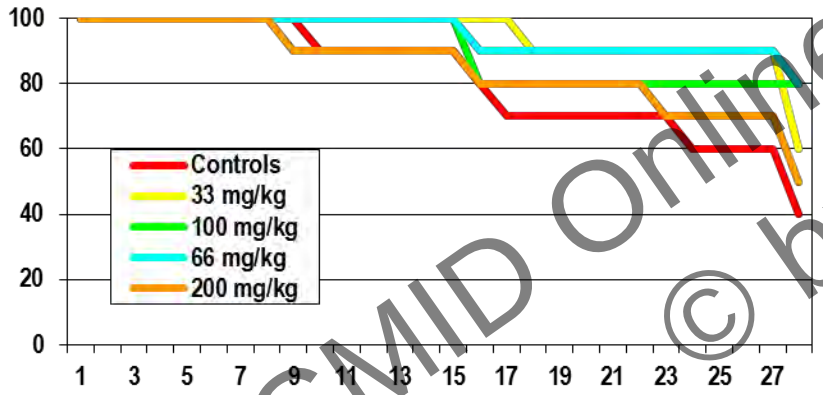
Poissy, AAC 2010

➲Prevents mortality at high dosing

➲ No bactericidal activity

Results: mutant GyrA D94G (MXF MIC = 4 mg/l)

% surviving mice depending on dosing



Log10 CFU after 1 month

Dosing (mg/kg/j)	33x3	100x1	66x3	200x1
Δ (log10 UFC)	+1	+1	+0,6	+1

Poissy, AAC 2010

➲ Does not prevent mortality
➲ No bactericidal activity

Conclusion 1

- In a murine model, Moxifloxacin retains partial activity against DNA gyrase mutants with MXFX MIC ≤ 2 mg/L

- Still true when included in a drug combination?

M & M

Strain	N mice sacrificed after X months treatment						Untreated controls	Total
	D-13	D0	M2	M3	M6	M6+3		
H37Rv	10	20	10	10	10	10	10	80
GyrB D500N	10	20	10	10	10	10	10	80
GyrA A90V	10	20	10	10	10	10	10	80
GyrA D94G	10	20	10	10	10	10	10	80
Total	40	80	40	40	40	40	40	320

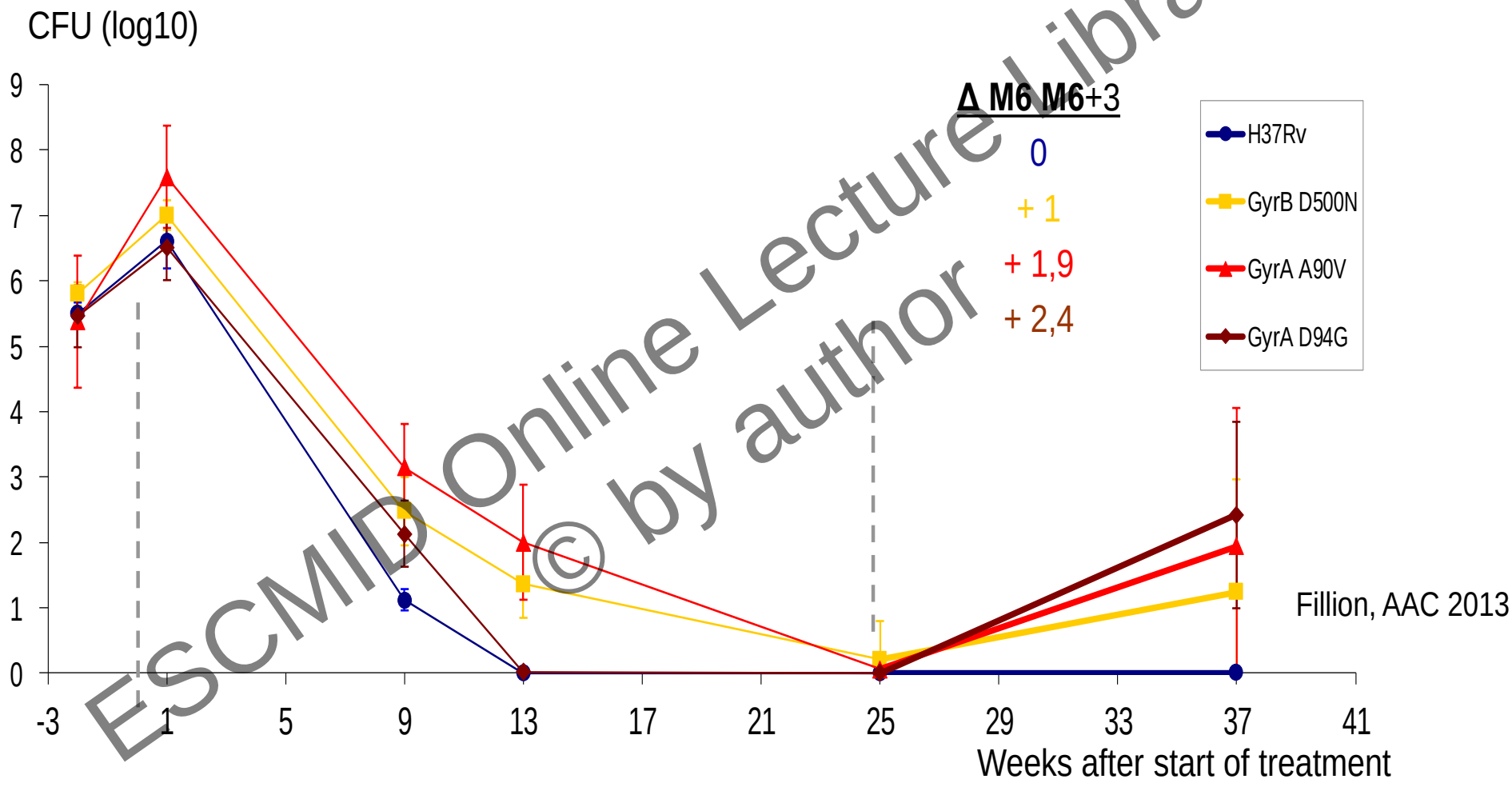
Amikacin 2 mois
Moxifloxacin 6 mois
Ethionamide 6 mois
Pyrazinamide 6 mois

Fillion, AAC 2013

Amikacin SC : 15 mg/kg # 6400 injections

Moxifloxacin : 100 mg/kg x 2 /j
Ethionamide : 50 mg/kg/j
Pyrazinamide : 150 mg/kg/j
27 000 gavages

Results : CFU M6+3



→ Sterilizing activity correlated with resistance level

Conclusion 2

- The impact of fluoroquinolone resistance is not ON/OFF
- In a murine model, the level of moxifloxacin resistance is correlated with the activity of a moxifloxacin containing MDR drug combination
- Supports the use of moxifloxacin in case of low-level resistance
- And in human?

Human data : Korea

- Jo, IJTLD 2014
  - MDR TB treatment
  - GTFX, MXFX, LVFX, dosing not specified
  - Results on 54 strains OFX-R (2 mg/L LJ) depending on MXFX susceptibility (≤2 mg/L LJ)

Table 5 Treatment outcomes of 54 patients with ofloxacin-resistant MDR-TB who received later-generation FQs

Treatment outcome	Moxifloxacin-susceptible group* (n = 20) n (%)	Moxifloxacin-resistant group† (n = 34) n (%)	P value
Total treatment duration, months, mean ± SD	22.8 ± 9.4	19.0 ± 11.2	0.207
Duration of FQ treatment, months, mean ± SD	21.8 ± 9.3	12.8 ± 11.4	0.008
Potentially active drugs, median [range]‡	2 [0–5]	3 [0–5]	0.465
WHO Group 5 drugs, median [range]§	1 [0–2]	1 [0–3]	0.865
Treatment outcomes			
Cured	12 (60.0)	13 (38.2)	0.121
Treatment completed	2 (10.0)	2 (5.9)	0.622
Treatment failure	2 (10.0)	15 (44.1)	0.014
Lost to follow-up	1 (5.0)	1 (2.9)	1.000
Not evaluated	1 (5.0)	2 (5.9)	1.000
Died	0	1 (2.9)	1.000
Currently on treatment	2 (10.0)	0	0.133
Treatment success	14 (70.0)	15 (44.1)	0.065

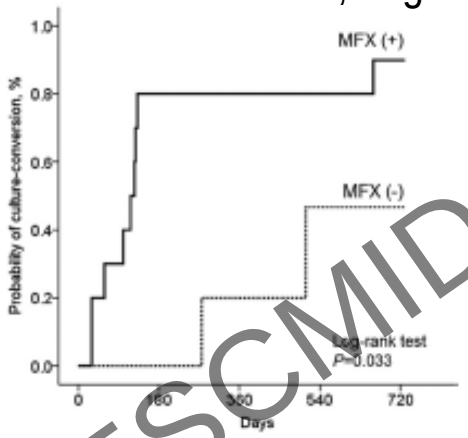
*Of these 20 patients, 19 received MFX and one received LFX.
†Of these 34 patients, 21 received MFX, 10 received LFX and 3 received gatifloxacin.
‡Later-generation FQ was not counted as an active drug in either group. WHO Group 5 drugs were excluded from this calculation.
§Includes linezolid, clofazimine, amoxicillin/clavulanate and clarithromycin.
MDR-TB = multidrug-resistant tuberculosis; FQ = fluoroquinolone; SD = standard deviation; WHO = World Health Organization; MFX = moxifloxacin; LFX = levofloxacin.

Confirms the 2 mg/L moxifloxacin breakpoint

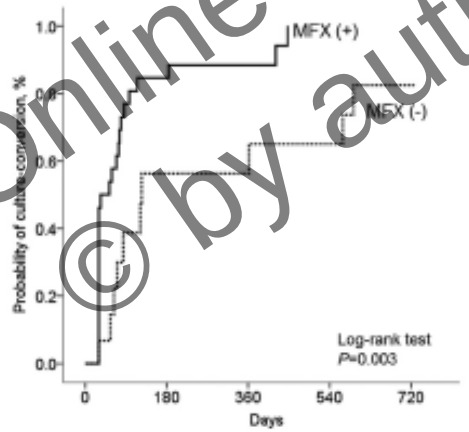
Human data : Taiwan

- Chien, AAC, 2016
  - MDR TB treatment
  - MXFX, dosing not specified
  - 81 patients

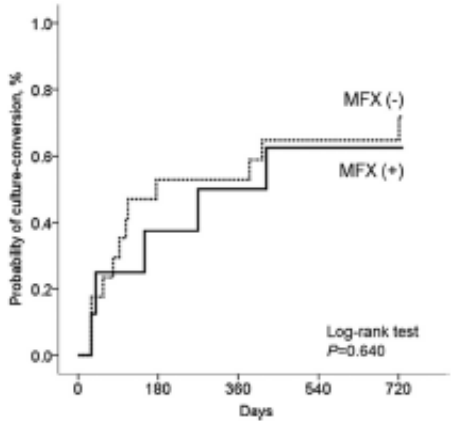
MXF MIC< 0,5mg/L



MXF MIC 1-2mg/L



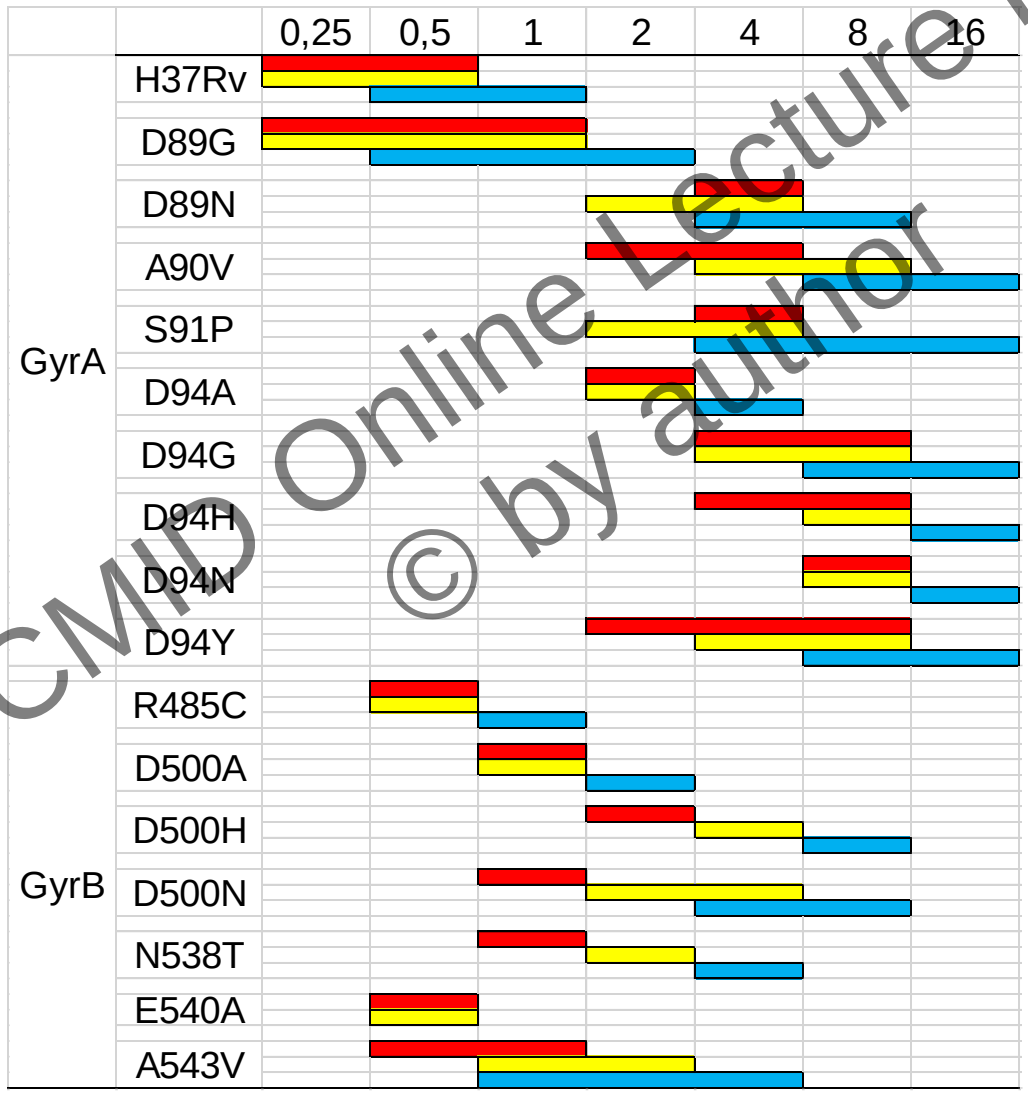
MXF MIC>2mg/L



Confirms the 0,5 and 2 mg/L moxifloxacin breakpoint

Can the phenotype be predicted by the genotype?

Genotype vs phenotype



MXFX
LVFX
OFX

D94 mutants : higher MXFX MICs (except D94A)

Bernard, JAC, 2016

Human data : Genotype

- Rigouts, JAC, 2016

Table 4. Summary of gatifloxacin treatment outcome stratified by *gyrA-gyrB* sequence result; only one pre-MDR-treatment isolate per patient included

			Failure/relapse		
gyrAB QRDRs	Mutation type	Cured	*n*	%	Total
Any *gyrAB* QRDR mutant		31	31	50.0	62
Any *gyrA* QRDR mutant		29	29	50.0	58
Any gyrA 94 mutant except 94Ala		*8*	*18*	*69.2*	*26*
Remaining gyrA mutants		*21*	*11*	*34.4*	*32*

- Chien, AAC 2016

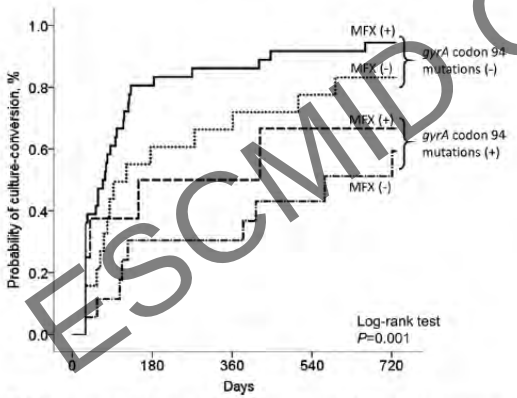


FIG 2 Kaplan-Meier plots and log rank test for probability of culture conversion. The Kaplan-Meier time-to-event analysis is based on treatment with or without MFX and the presence of mutations at codon 94 in the *gyrA* gene.

GyrA 94 mutants (except D94A) have worse prognosis than others

Conclusion 3

- Low-level moxifloxacin resistance MDR strains (≤2 mg/L MIC)
  - Have improved prognosis if MFXF is maintained in the drug regimen
  - Can be predicted by genotype
- Moxifloxacin susceptibility testing at 2 levels endorsed by WHO
  - 0,5 and 2 mg/L
- What about
  - other fluoroquinolones?

Human data : Gatifloxacin

- Aung, IJTLD 2014
  - Gatifloxacin, (800 mg/d >50kg) ethambutol, pyrazinamide, clofazimine on whole duration
  - Initial phase 4 months : kanamycin, prothionamide and isoniazid
  - Total lenght = 5 months after end initial phase (at least 4 months and then up to sputum culture negativity)

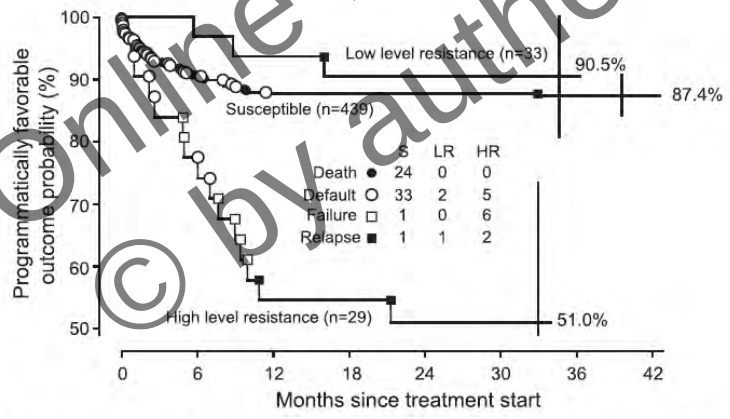


Figure 3 Programmatically favorable treatment outcome probability derived from a Cox's proportional hazard model among 501 patients, stratified by initial fluoroquinolone susceptibility test result, adjusted for age and sex. S = susceptible to ofloxacin and/or GFX at the standard critical concentration; LR = low-level resistance (GFX MIC 0.5–1.0 mg/l); HR = high-level resistance (GFX MIC ⩾2 mg/l); GFX = gatifloxacin; MIC = minimum inhibitory concentration.

GTFX high dosing → same prognosis as susceptible strains

if GTFX MIC≤1 mg/L

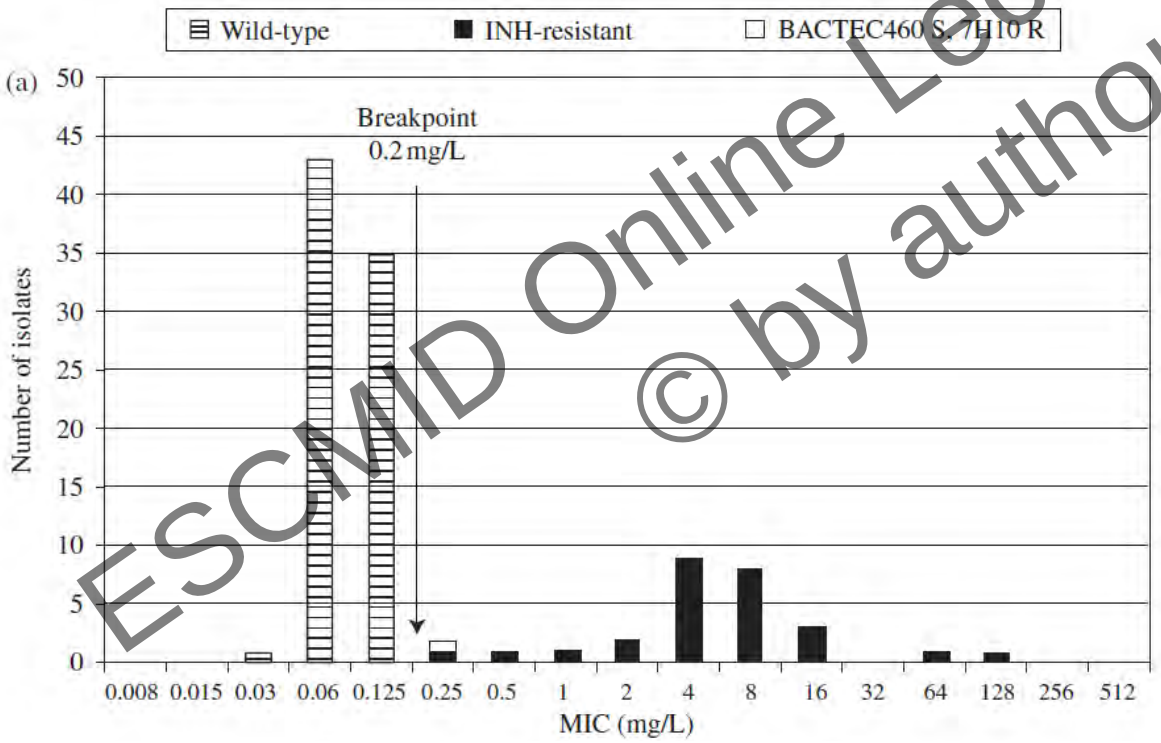
Conclusion 4

- Gatifloxacin high dosing also promising but toxicity concerns

- What about other antibiotics?

Isoniazid, low-level resistance

- Schön JAC 2009, Donald JID 2004

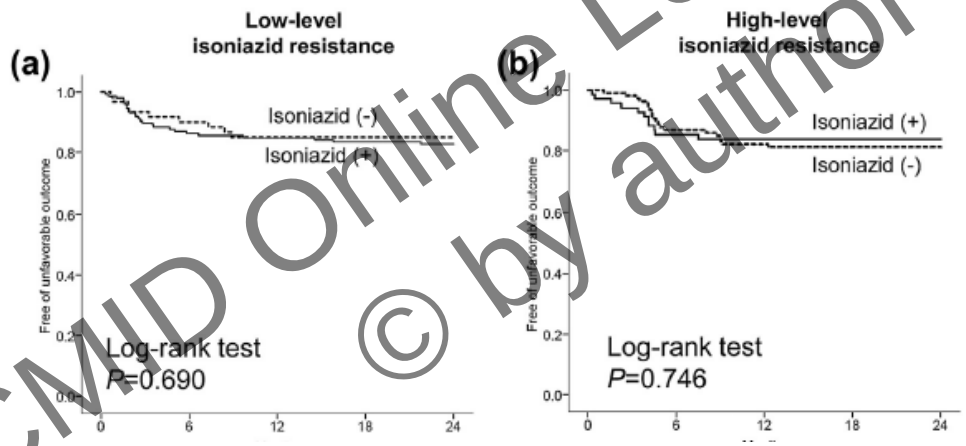


Serum level (mg/l)	EBA log10)
0-0,5	-0.034
0,5-1	0.342
1-,1,5	0.455
1,5-2	0.390
2-3	0.609
3-6	0.633
≥6	0.526

Low expectations

Combined therapy against isoniazid resitant strains

- Chien, CMI, 2015



Not in favor of in vivo activity of isoniazid against low-level resistant strains

Conclusion 5

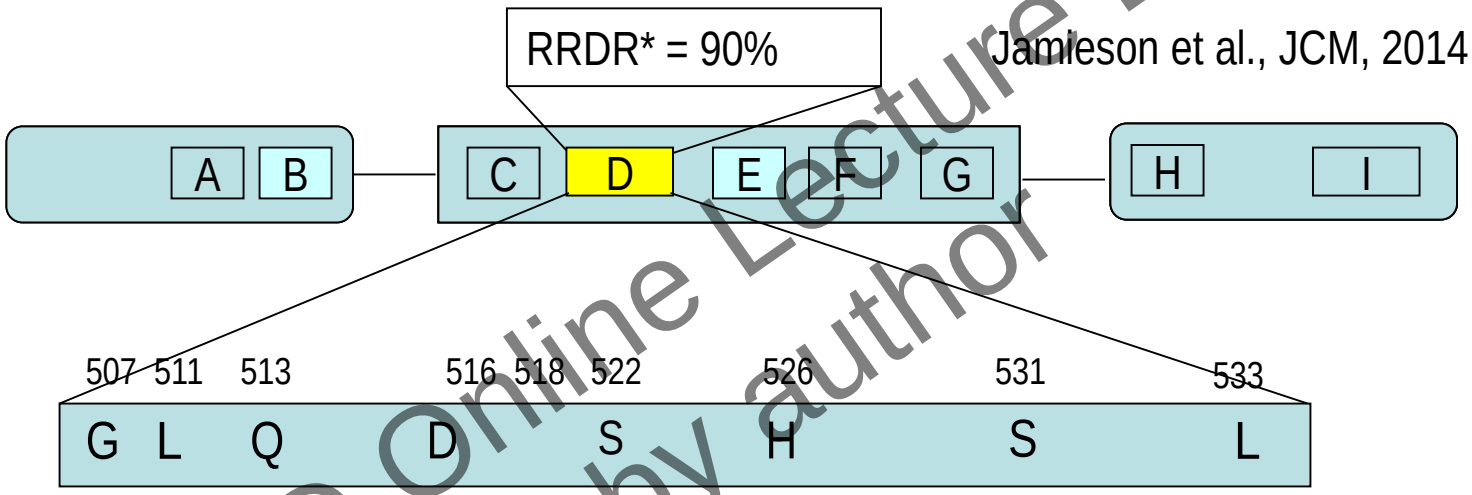
- Activity against low-level resistant mutants may not stand true for all drugs

Various levels of resistance: 2 sides of a same coin

the dark side : reduced activity

when low-level resistance is missed

Rifampin : different levels of resistance



L
D
W

High MIC
(>20mg/L)

V L Y

Intermediate MIC
(1 à 20 mg/L)

L
N

P

Low MIC
(<1mg/L)

P

Normal MIC

genotype/phenotype discrepancy

Mme N:

- July 2012 : Pulmonary TB

→ Isoniazid-R

MTBDR*plus* : *katG* S315T, *rpoB* WT

07/2012 HRZE (2 months)

- Octobre 2012 : fracture right foot, unfavorable evolution, fistula

09/2012 RZE (2 months)
Then RZ (5 months)

04/2013 Stop treatment

- Octobre 2013 : pain in foot, multiple abcesses

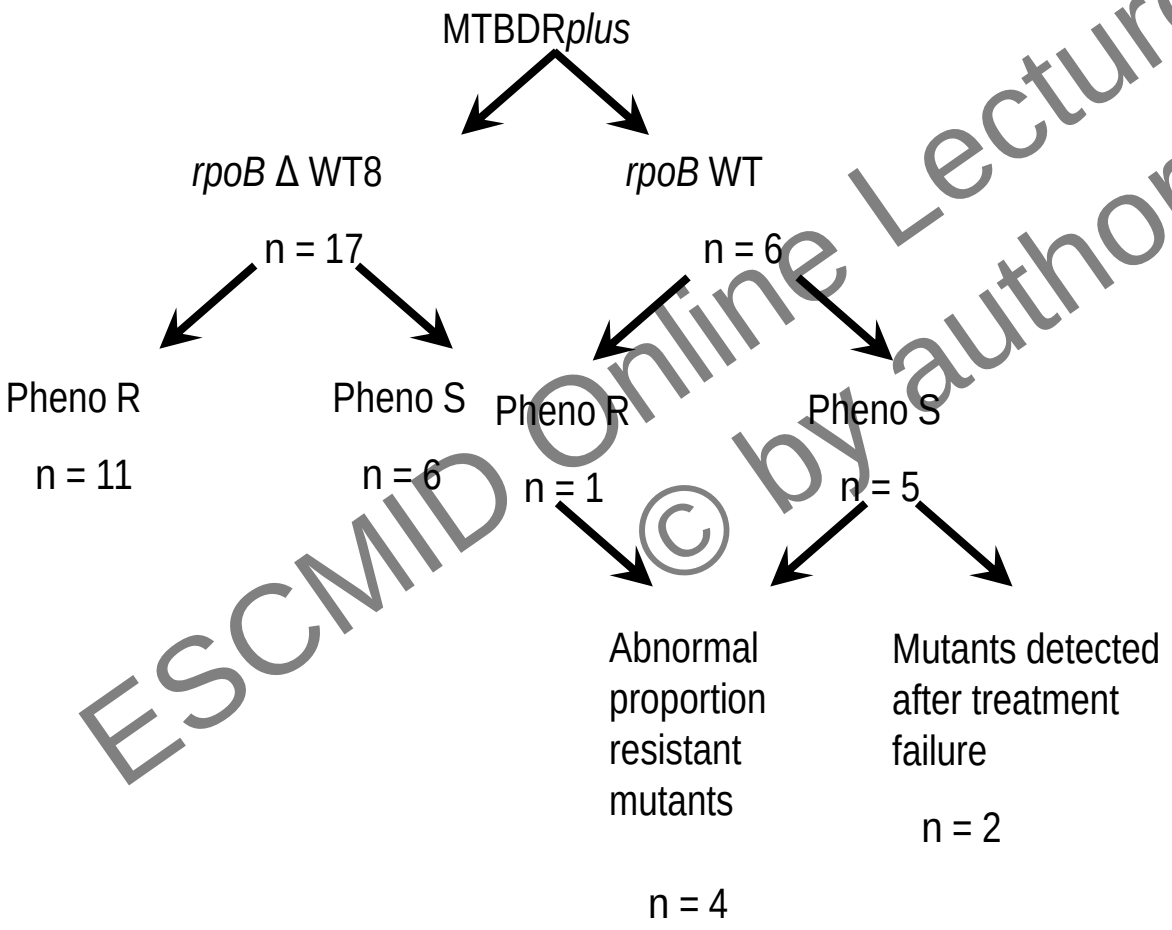
- January 2014 : surgery, C+ *M. tuberculosis*
  - MTBDR*plus* : *katG* S315T, *rpoB* delta WT8; sequencing *rpoB* : mutation L533P
  - MDR strain

Test repated on 2012 strain : *rpoB* sequencing : L533P mutation

→ The 2012 strain was MDR!!!

Retrospective study : strains received at NRC since 2005

- 23 L533P strains
- 75% R-isoniazid



→ MTBDR*plus* detects L533P in ¾ cases

→ Phenotypic DST detects resistance in ½ cases

Rifampin low-level resitance

Phenotypic detection
LJ and MGIT

tuberculosis isolates

rpoB mutation type	No. of strains	LJ result (MIC_{99} [μg/ml])	No. (%) of isolates determined to be r	
			LJ (at MIC_{99})	MGIT (at 1 μg/
511Pro	6	80–640	6 (100)	0
513Lys	2	>640	2 (100)	2 (100)
513Pro	3	>640	3 (100)	3 (100)
516Phe	4	>640	4 (100)	4 (100)
516Val	15	320–>640	15 (100)	15 (100)
516Tyr	6	320–>640	6 (100)	0
522Gln	11	320–>640	11 (100)	8 (73)
522Leu	2	>640	2 (100)	2 (100)
526Asp	11	≥640	11 (100)	8 (73)
526Arg	5	>640	5 (100)	5 (100)
526Leu	10	≥640	10 (100)	6 (60)
526Tyr	14	≥640	14 (100)	10 (71)
526Asn	5	80–>640	5 (100)	1(20)
531Leu	10	≥640	10 (100)	10 (100)
531Trp	4	>640	4 (100)	4 (100)
533Pro	14	160–>640	14 (100)	0
572Phe	6	40–>640	6 (86)	0

rpoB mutation(s)	RMP susceptibility at indicated MIC (μg/ml) determined by:								
	LJ medium						MGIT 960		
	1.25	2.5	5	10	20	40	0.25	0.5	1
L533P	R	R	S	S	S	S	R	S	S
D516Y, N518D	B	S	S	S	S	S	S	S	S
L533P	R	R	R	S	S	S	R	S	S
H510H, D516Y	R	R	R	R	R	S	R	R	S

Rigouts, JCM 2013
Andres, AAC 2014

Some *rpoB* mutations confer low-level resistance are difficult to detect phenotypicaly and are associated with treatment failure

Conclusion 6

- Low-level resistance poorly detected by phenotypic tests can have negative impact on treatment outcome

Conclusion

- Genotypic diagnosis of drug resistance sheds light on the diversity of levels of resistance
- Low-level resistant mutant (i.e with elevated MIC remaining below peak serum level of antibiotic)
  - Are an opportunity to overcome resistance with high dosing of antibiotic
    - Demonstrated clearly for fluoroquinolones
    - May stand true for other drugs : rifamycines, bedaquiline, etc
  - Increase risk of failure if missed by phenotypic diagnosis
    - Demonstrated clearly for rifampin
    - May stand true for other drugs : fluoroquinolones, etc
- Requires a systematic *in vitro* and *in vivo* work of classification of these different mutants

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