

# Cross Resistance of Antituberculosis Drugs



Anti-TB drug	Gene mutation
Isoniazid	<i>katG, inhA, ahpC, KasA</i>
Rifampin	<i>rpoB</i>
Pyrazinamide	<i>pncA</i>
Ethambutol	<i>embB</i>
Streptomycin	<i>rpsL, rrs</i>
Flouroquinolones	<i>gyrA, gyrB</i>
Ethionamide	<i>inhA, etaA/ethA</i>
Cycloserine	<i>alrA, Ddl</i>
PAS	<i>unknown</i>



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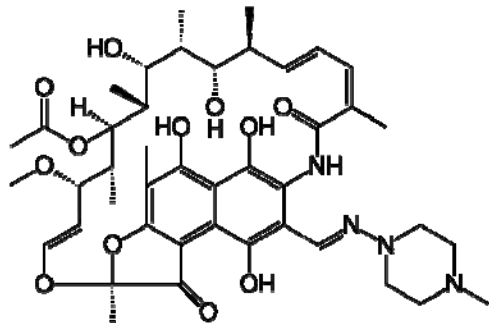
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# Cross-Resistance Between Anti-tuberculosis Drugs

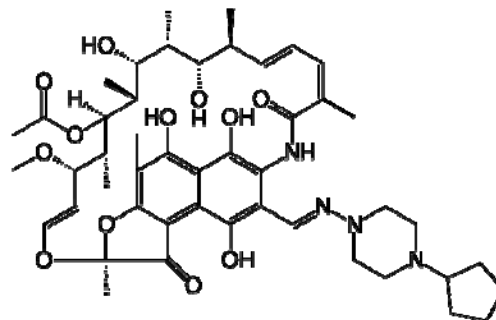
<i>Drug</i>	<i>Cross Resistance</i>
Rifampin	High level cross-resistance with other rifamycins
Fluoroquinolones	Variable cross-resistance; some newer generation drugs remain susceptible when lower-generation drugs are resistant
Aminoglycosides and polypeptides	Amikacin and kanamycin have high cross-resistance Capreomycin and viomycin have high cross-resistance Variable cross-resistance between other drugs
Prothionamide and ethionamide	High level cross-resistance Variable cross-resistance with isoniazid
Thioacetazone	Variable and low cross-resistance to isoniazid, ethionamide and PAS

# Rifamycins

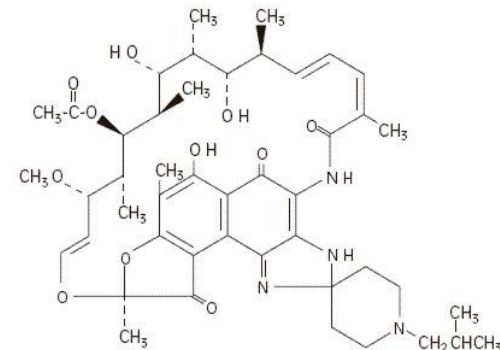
Rifampin



Rifapentine



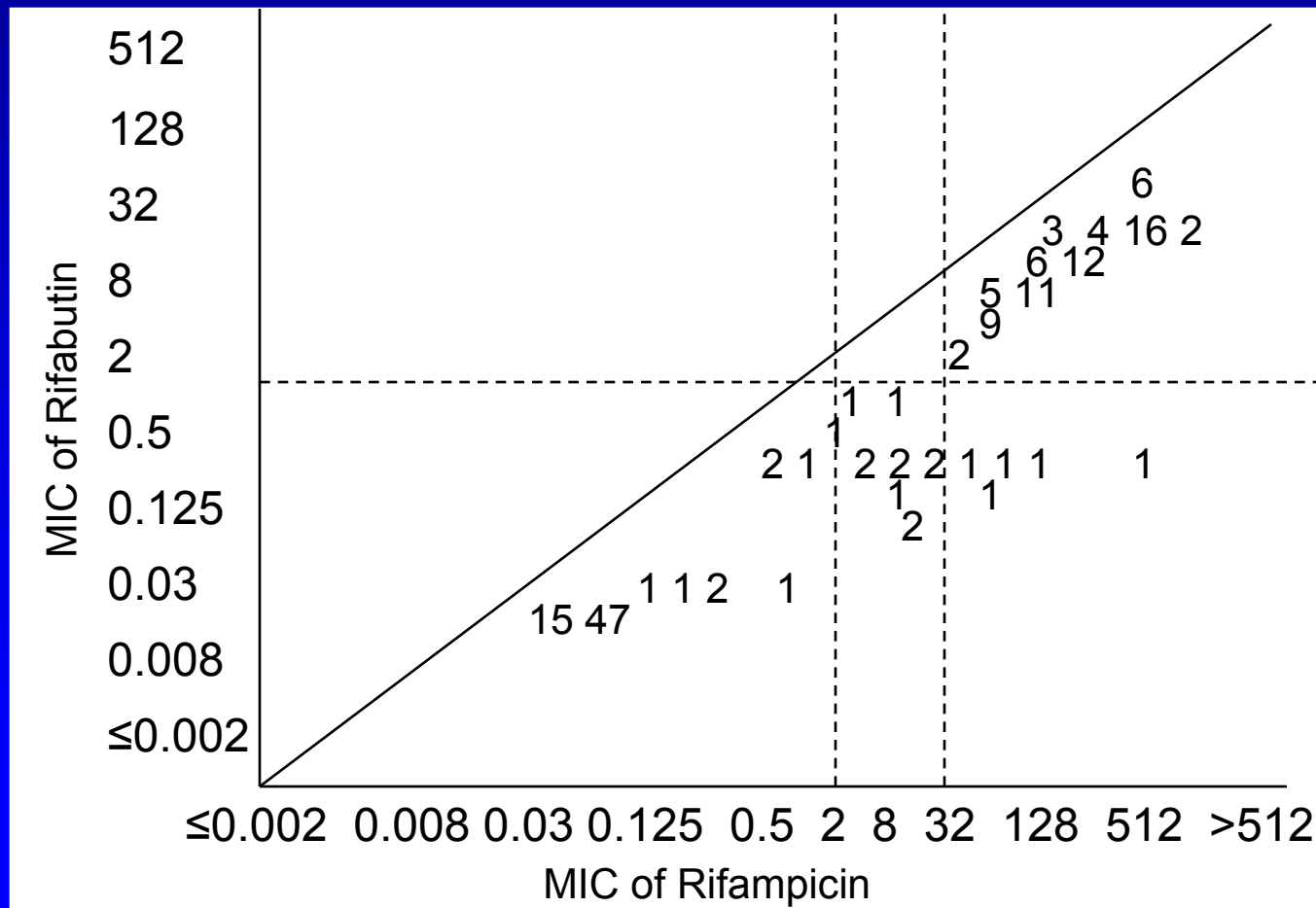
Rifabutin



Resistance - mutation in the 81 bp region of the RNA polymerase  $\beta$ -subunit (*rpoB*) gene

# Correlation Between MICs of Rifampicin and Rifabutin

163 *Mycobacterium tuberculosis* strains



93 rifampin-resistant strains (MIC≥2)  
 • 17 (5%) were susceptible to rifabutin (MIC ≤1)

# Rifamycin MICs for Clinical Isolates of Mtb Mutant *rpoB* Alleles

29 rifampin-resistant strains: 23 cross-resistant (18 mutations)  
6 (20%) susceptible to rifabutin (5 mutations)

<i>Strain</i>	<i>Mutated AA</i>	<i>MIC *ug/ml</i>		
		<i>Rifampin</i>	<i>Rifabutin</i>	<i>Rifapentine</i>
1	511 (Leu → Pro)	2	0.5	1
2	516 (Asp → Val)	32	< 0.5	32
3	516 (Asp → Val)	32	0.5	32
4	516 (Asp → Tyr)	2	< 0.5	8
5	519 (Asn → Lys)	32	0.5	32
6	522 (Ser → Leu)	32	0.5	32

# Cross-resistance between Rifampicin and Rifabutin

- Site: Izmir, Turkey
- 41 rifampicin-resistant strains of *M. tuberculosis*
- DST by proportion method on 7H10 media
  - » Critical concentration: rifabutin 0.5mg/L, rifampin 1.0mg/L
- Results:
  - » 35 isolates with rifampin MICs  $\geq 32$  mg/L were also rifabutin-resistant
  - » 6 (14%) isolates with rifampin MICs 2-16 mg/L were susceptible to rifabutin (MIC  $\leq 0.5$  mg/L)

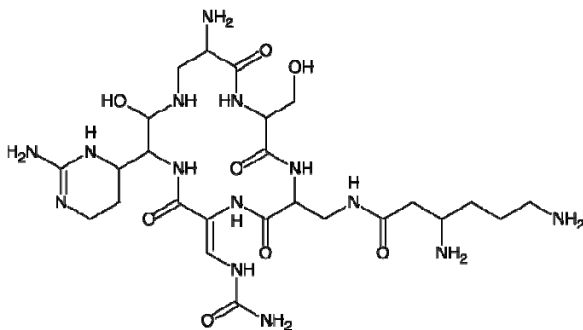
# In vitro Activity of Rifabutin Against Rifampin-resistant *M. tuberculosis*

No. Strains	Rifampicin	Rifabutin	Sequences	AA Change
1	64	2.5	531 TCG → TTG	Ser → Leu
1	32	5	514 515 ins CGG	Arg
1	32	5	531 TCG → TTG	Ser → Leu
1	32	1	531 TCG → TTG	Ser → Leu
2	16	0.5	516 GAC → TAC	Asp → Tyr
1	8	0.5	516 GAC → GTC	Asp → Val
1	8	0.125	515 ATG → ATC 533 CTG → CCG	Met → Ile Leu → Pro
1	2	0.5	533 CTG → CCG	Leu → Pro
1	2	0.0625	526 CAC → TGC	Leu → Pro

# Aminoglycosides and Polypeptides

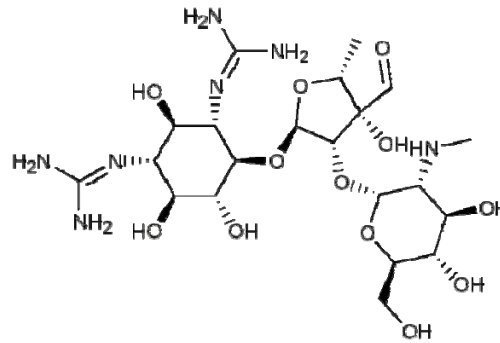
## Polypeptides

### Capreomycin (Viomycin)

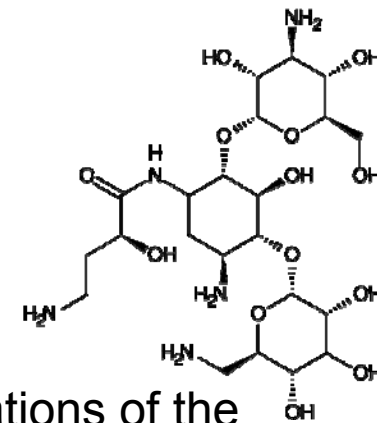


## Aminoglycosides

### Streptomycin



### Amikacin (Kanamycin)



- » Streptomycin - resistance is associated with alterations of the ribosomal target site resulting from mutations in the *rspL* gene of the S12 ribosomal protein or *rrs* gene of the 16S rRNA
- » Capreomycin - mutations in 16S rRNA gene (*rrs*) or mutation in *tlyA* gene, encoding a putative rRNA methyltransferase

# Cross-Resistance in *M. tuberculosis* to KAN, CAP, VIO

<i>Strains</i>	<i>Drug Concentration (µg/ml)</i>			
	<i>SM</i>	<i>VM</i>	<i>KM</i>	<i>CM</i>
SM - R	>40	3	3	4
	>40	4	4	6
	ND	ND	ND	ND
	>40	5	4	6
VM - R	<1	40	4	>40
	<1	>40	8	>40
	1	>40	>40	>40
	<2	40	10	>40
CM - R	<1	>40	4	>40
	2	>40	6	>40
	1	>40	>40	>40
	4	>40	15	>40
KM - R	<1	5	>40	40
	3	>40	>40	>40
	ND	5	>40	15
	2	8	>40	>40

# Sequencing Results in 10 Clinical Isolates with Different Resistance Patterns

<i>Clinical isolates</i>	<i>KM (MIC)</i>	<i>AK (MIC)</i>	<i>SM*</i>	<i>Mutations in the rrs gene</i>
1	S (1)	S ( $\leq 1$ )	S	None
2	S (1)	S ( $\leq 1$ )	R	None
3	S (4)	S ( $\leq 1$ )	R	None
4	R (8)	S ( $\leq 1$ )	R	None
5	R (16)	S ( $\leq 1$ )	R	None
6	R (32)	S ( $\leq 1$ )	S	C517T
7	R (32)	S ( $\leq 1$ )	R	None
8	R (32)	S ( $\leq 1$ )	R	None
9	R (64)	R (8)	S	C516T
10	R (>256)	R (>256)	R	A1400G

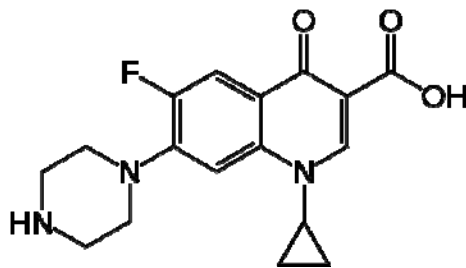
\*Drug concentration, 4.0 ug/ml

# Mutations and MICs in DR Clinical Isolates

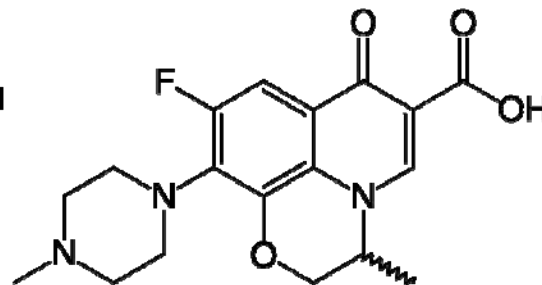
Clinical isolate	<i>rrs</i> mutation	CAP	VIO	KAN	AK
1	A1401G	20	≤10	>80	>64
2	A1401G	20	≤10	>80	>64
3	A1401G	20	≤10	>80	>64
4	A1401G	20	≤10	>80	>64
5	A1401G	40	≤10	>80	>64
6	A1401G	20	≤10	>80	>64
7	A1401G	40	≤10	>80	>64
8	A1401G	80	≤10	>80	>64
9	A1401G	20	≤10	>80	>64
10	A1401G, A514C	20	≤10	>80	>64
11	A1401G, T1239C, A514C	20	≤10	>80	>64
12	C1484T, C1105G	>160	>80	>80	>64
13	C517T	80	≤10	10	≤4
14	C517T	20	≤10	40	≤4
15	None	20	≤10	40	8
16	None	80	80	40	≤4

# Fluoroquinolones

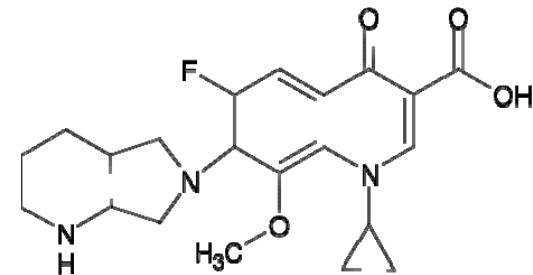
Ciprofloxacin



Ofloxacin  
(levofloxacin)



Moxifloxacin  
(gatifloxacin)



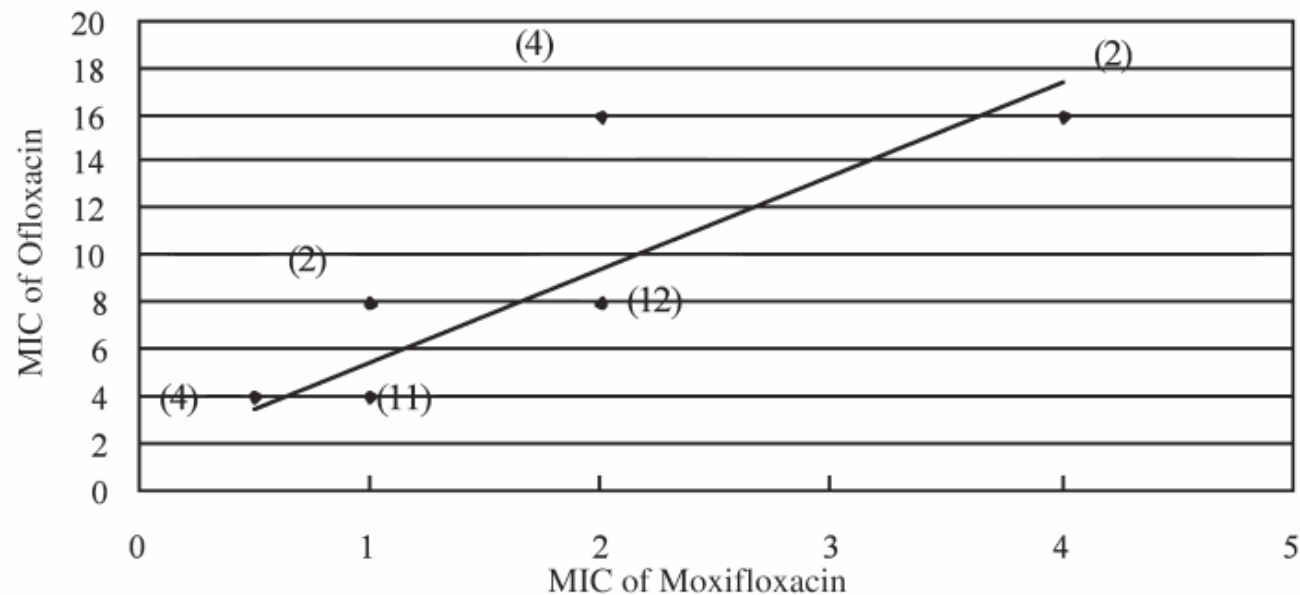
Resistance - mutations in *gyrA* and *gyrB*

# Stepwise Decrease in Moxifloxacin Susceptibility

- Site: Hong Kong TB Reference Lab
- 132 MDR-TB strains from patients, 1999-2003
  - » 108 were ofloxacin susceptible
  - » 24 were ofloxacin resistant (break-point >2.4 mg/L)
  - » 11 additional nonMDR ofloxacin resistant strains
- MGIT for DST with concentrations of 0.5, 1.0, 2.0, 4.0, 8.0 and 16.0 mg/L
- DNA sequencing of gyrase A was performed

# Correlation Between MICs (mg/L) of Ofloxacin and Moxifloxacin

35 ofloxacin-resistant strains of *M. tuberculosis*



Linear regression:  $y = 4.02x + 1.33$ ;  $R^2 = 0.62$

( ): Number of strains at that MIC data point

MIC values of moxi were at least 4-fold that of oflox

# Correlation Between Type of *gyrA* Mutation and MIC

Stepwise decline in MOX susceptibility correlated with OFX susceptibility

<i>gyrA</i> mutation	Number of strains	MIC (mg/L)					
		OFX4 MOX0.5	OFX4 MOX1	OFX8 MOX1	OFX8 MOX2	OFX16 MOX2	OFX16 MOX4
Ala-90Val+Pro-102His	2	1	1				
Asp-94Ala	5	1	3	1			
Ala-90Val	8	2	4	1		1	
Ser-91Pro	1		1				
Ala-126Arg	2		1		1		
Asp-94His	1				1		
Asp-94Gly	12				8	3	1
Asp-94Tyr	2				1		1
No specific mutation	2		1		1		
<b>Total</b>	<b>35</b>	<b>4</b>	<b>11</b>	<b>2</b>	<b>12</b>	<b>4</b>	<b>2</b>

Asp-94Gly accounted for 12 of the strains with high MICs for oflox and moxi

# Comparative Susceptibilities of Fluoroquinolones in 138 Clinical Isolates

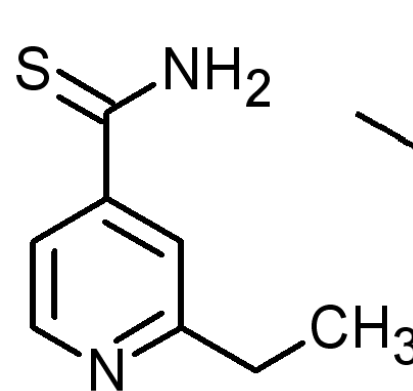
MIC (mg/L)	% of Strains			
	Moxifloxacin		Ofloxacin	
	(+)R, (-)gyrA (n=23)	(+)R, (+)gyrA (n=32)	(+)R, (-)gyrA (n=23)	(+)R,(+) gyrA (N=32)
≤0.125	17			
0.25	43			
0.5	30			
1	9	31		
2		53	83	3
4		13	13	19
8		3	4	28
16				38
>16				13

# Isoniazid and Amides

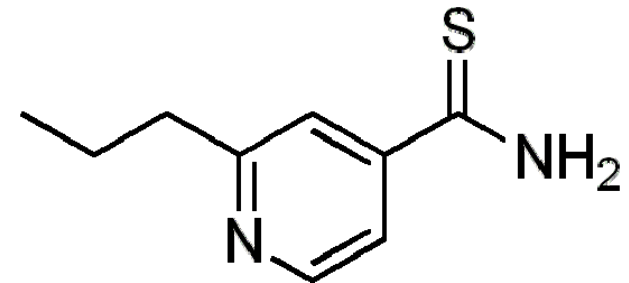
Isoniazid



Ethionamide



Protionamide



- » mutation in *inhA* structural gene and within *inhA* promoter gene
- » mutations in *katG*, particularly at codon 315, confer INH but not ETH resistance

# Cross-resistance Between Isoniazid and Ethionamide

“Many wild strains of *Mycobacterium tuberculosis* contains mutants of an extremely low degree of resistance against isoniazid. These mutants are highly resistant to ethionamide, whereas the far more common mutants showing high degrees of isoniazid resistance are fully susceptible to ethionamide.”

George Canetti 1965

# Mutations in *ethA* and *inhA* and Association with ETH-resistance

ETH MIC	No. isolates	<i>ethA</i> only	<i>inhA</i> ORF or promoter	<i>ethA</i> and <i>inhA</i>	No mutations
> 200	17	5	9	3	0
200	2	1	0	1	0
100	5	1	4	0	0
50	5	2	0	2	1
25	1	0	1	0	0
20	4	0	4	0	0
10	4	0	3	0	0
5	0	0	0	0	0
2.5	0	0	0	0	0
< 2.5	3	0	1	0	2
Total	41	9	23	6	3

# Exclusive Mutations Related to INH and Ethionamide

24 clinical isolates from South Korea

<i>Resistance to:</i>	<i>No. isolates</i>	<i>Mutation in 94th codon/regulatory region</i>	<i>N (%)</i>
INH and ETH	12	– /C to T	12 (100%)
INH only	12	– / – – /C to T	10 (83.3%) 2 (16.7%)
H37Rv	1	– / –	0

# Summary

- Rifamycins:
  - » Usually complete cross-resistance with rifampin and rifapentine
  - » Variable cross-resistance between rifampin and rifabutin (5-20%)
- Aminoglycosides:
  - » Usually complete cross-resistant between amikacin and kanamycin
  - » Usually complete cross resistance between capreomycin and viomycin
  - » Variable cross-resistance between amikacin, kanamycin and polypeptides

# Summary

- Fluoroquinolones:
  - » Variable cross-resistance with generally better activity in later generation FQNs
- Isoniazid and Thioamides:
  - » Usually complete resistance between ethionamide and prothionamide
  - » Cross-resistance between INH and thioamides with certain mutations in *inhA*

# Thank You!

