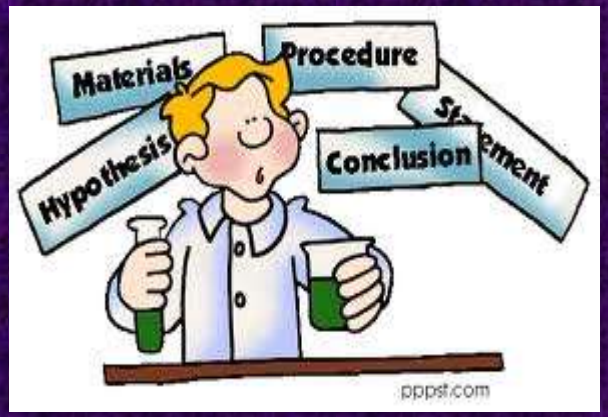
Investigation of cross-resistance/ tolerance to anti-TB drugs in Rifampicin resistant clinical *Mycobacterium tuberculosis* isolates



By
Miss Caroline Pule

Promoter: Prof. TC Victor

Co-promoter: Dr. GE Louw & Prof. RM Warren

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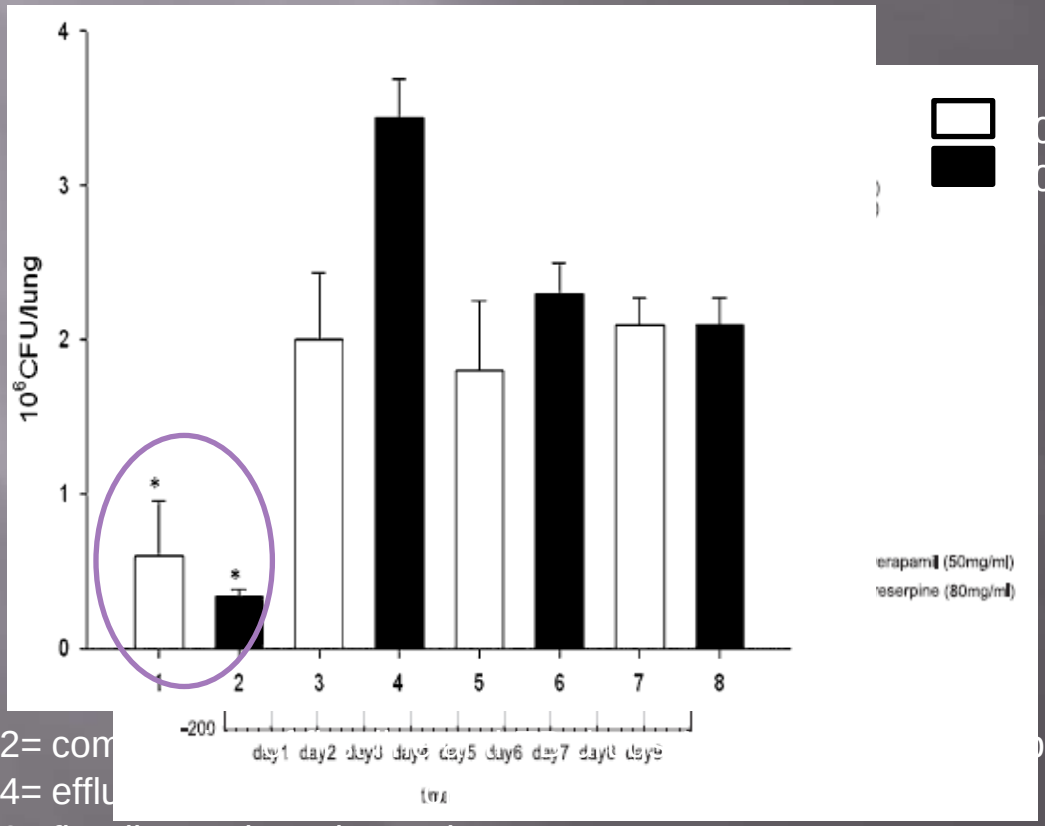
Central Dogma

Anti-TB drugs	Target genes
INH	*katG, InhA, ahpC, kasA, ndh*
RIF	*rpoB*
PZA	*pncA*
EMB	*embCAB*
STR	*rpsL,rrs,gidB*
FQ	*gyrA,gyrB*
KAN, AMI	*rrs*
CAP, VIO	*rrs,tlyA*
ETH	*InhA, ethA, ethR*

Introduction

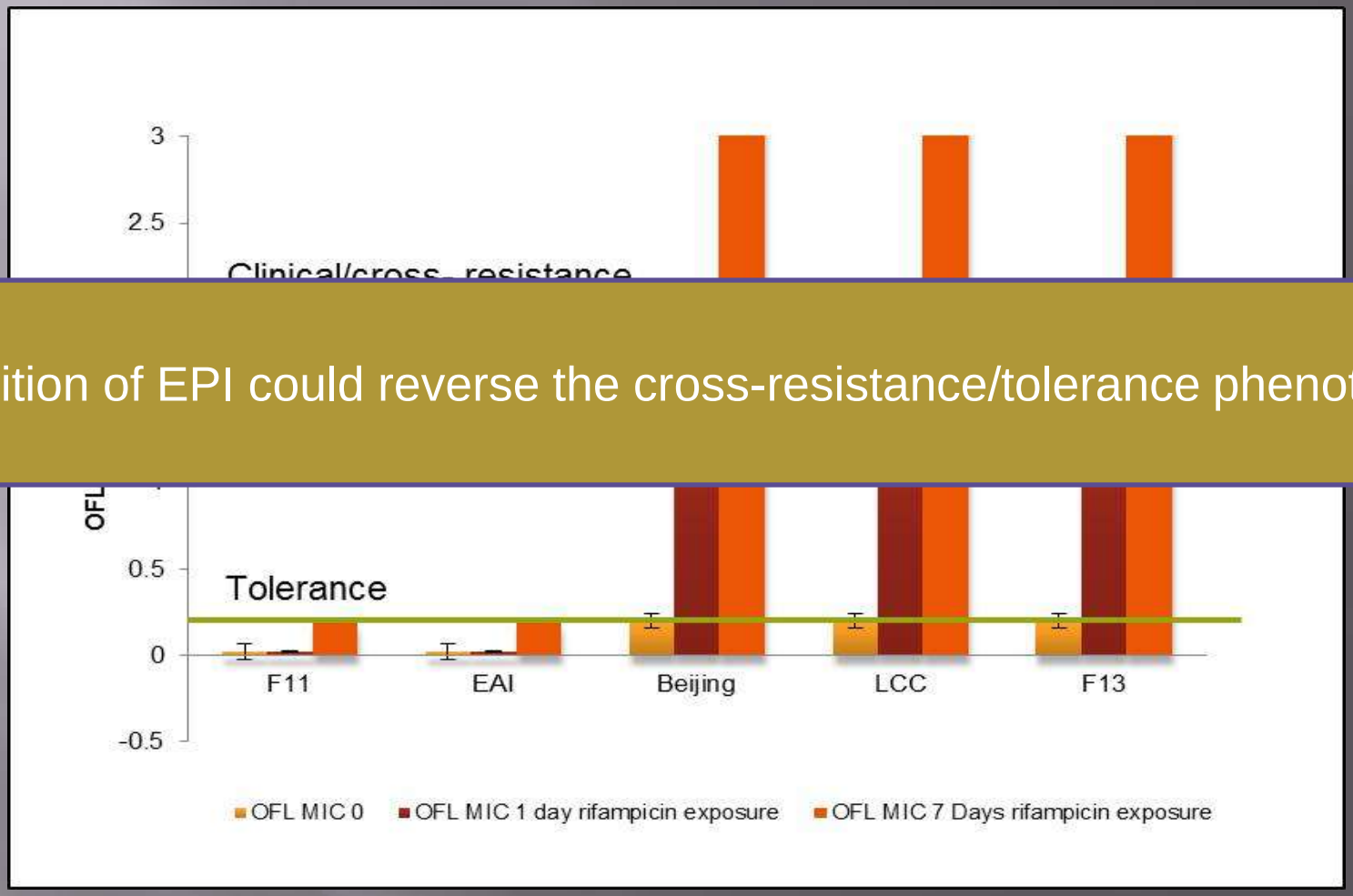
Rifampicin Reduces Susceptibility to Ofloxacin in Rifampicin-resistant *Mycobacterium tuberculosis* through Efflux

Gail E. Louw[1], Robin M. Warren[1], Nicolaas C. Gey van Pittius[1], Rosalba Leon[2], Adelina Jimenez[2], Rogelio Hernandez-Pando[3], Christopher R. E. McEvoy[1], Melanie Grobbelaar[1], Megan Murray[4], Paul D. van Helden[1], and Thomas C. Victor[1]



1;2= com… …itor
3;4= efflu…
5;6= first-line anti-TB drugs alone
7;8= saline

Introduction



Addition of EPI could reverse the cross-resistance/tolerance phenotype

Cross-resistance: conferred clinical resistance to Ofloxacin

Tolerance: did NOT confer clinical resistance but grew in the presence of Ofloxacin

Knowledge Gap!!

- Does rifampicin exposure also induce cross-resistance/tolerance to other anti-TB drugs that are structurally related/unrelated?
- Is this mechanism (efflux) of cross-resistance/tolerance a general phenomenon ?

Hypothesis

Exposure of rifampicin mono-resistant clinical *M. tuberculosis* isolates to rifampicin induces cross-resistance/tolerance to structurally related and unrelated anti-TB drugs by an efflux mechanism.

Objectives

- **Objectives**:
  - To determine the **baseline MIC** of ten anti-TB drugs in rifampicin mono-resistant *M. tuberculosis* clinical isolates with the same *rpoB*531 (Ser to Leu) mutation.
  - To determine whether EPI change the **baseline MIC** of any of the anti-TB drugs before and after exposure to rifampicin.

Strain selection

- rifampicin mono-resistant isolates
- *rpoB531* (Ser to Leu)

Family (n)	Spoligotype pattern	Spoligotype
LCC (n= 1)		118
Beijing (n= 4)		2
Haarlem (n= 1)		3
F13 (n= 1)		18
Unclassified (n = 1)		214

Experimental approach..(aim 1)

To determine the **baseline MIC** of the ten anti-TB drugs in rifampicin mono-resistant isolates

Critical concentration (CC)

Sensitive

Resistant

growth control (1:100)

Rifampicin mono-resistant strain culture in MGIT media

Increasing drug concentrations

culture and exposure to specific drug concentrations at CC and lower concentrations

Minimum inhibitory concentration (MIC)

(INH,ETH, OFL,MOXI,CIP,STR,CAP,AMI, EMB,PZA)

Results and Discussion (aim 1)

Isolates	Family	INH	STR	EMB	PZA	CIP	OFL	MOXI	CAP	AMI	ETH
		0.1- 0.4	1.0	5.0	100	1.0	2.0	0.25	2.5	1.0	5.0/12.5
R160	LCC	0.05	0.1	2.5	50	0.1	0.3	0.04	0.6	0.25	1.5
R376	Haarlem	0.05	0.1	0.5	200	0.1	0.3	0.06	0.6	0.25	0.6
R637	Beijing	0.05	0.1	2.5	50	0.25	0.3	0.04	0.6	0.25	1.0
R721	Beijing	0.05	0.1	2.5	150	0.1	0.3	0.04	0.6	0.1	> 12.5
R810	Beijing	0.1	0.25	2.5	200	0.25	0.3	0.06	1.0	0.25	>12.5
R966	Beijing	0.05	0.1	0.5	50	0.1	0.3	0.04	0.4	0.1	> 12.5
R1035	F13	0.05	0.25	0.5	50	0.1	0.3	0.04	0.6	0.25	>12.5
R458	Uncl.	0.05	0.1	2	100	0.25	0.3	0.04	0.6	0.25	1.25

Isolates

Drug name

Family name

Critical Conc.(µg/ml)

MIC (µg/ml)

http://www.who.int/tb/publications/2008/who+mtb-2008-392/en/index.html

Experimental approach..(aim 2)

To determine whether EPI change the **baseline MIC** of any of the anti-TB drugs before and after exposure to rifampicin

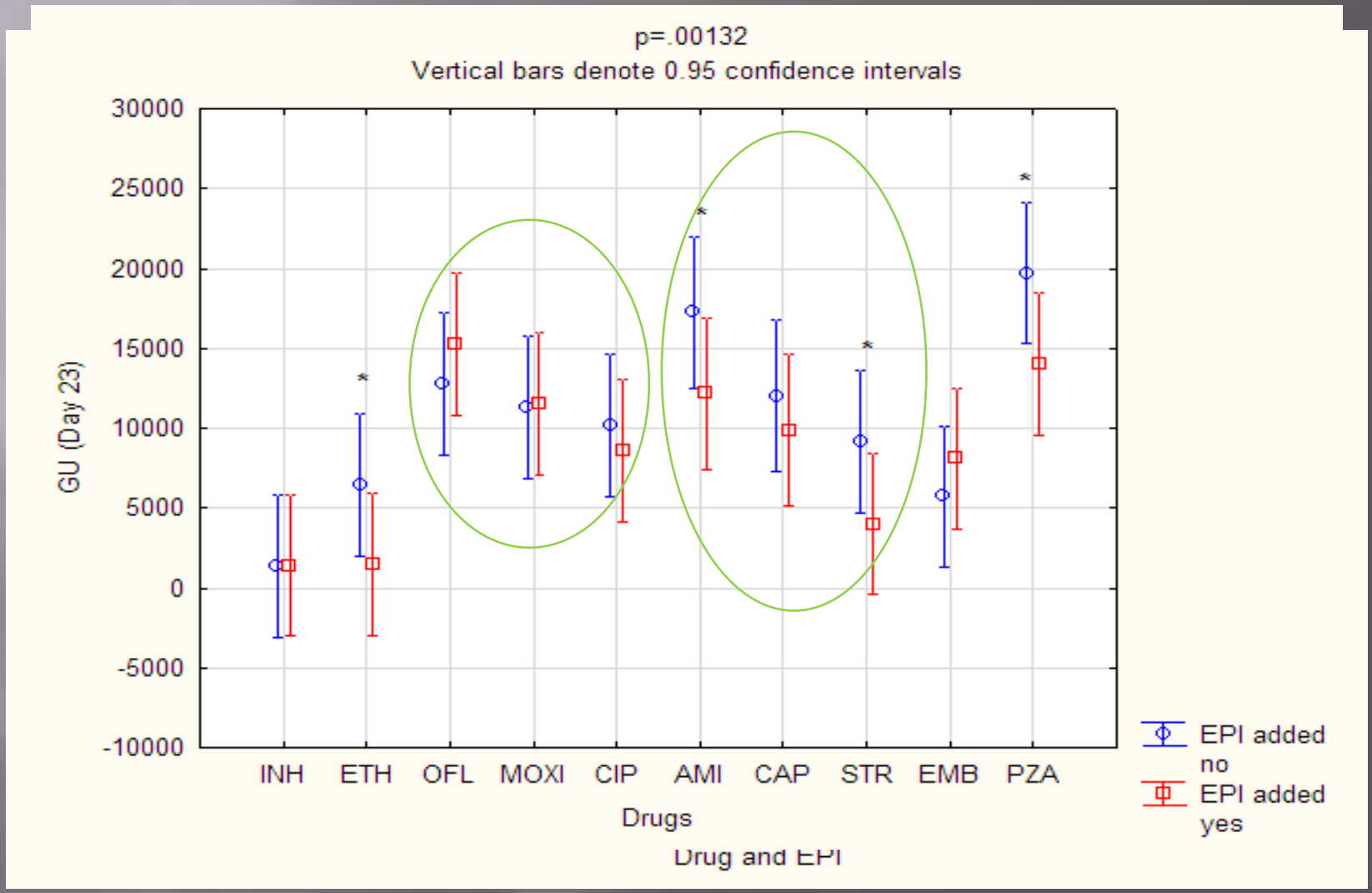
Rifampicin mono-resistant isolates (*rpo*B531)

- Initial **baseline MIC** determination (1st and 2nd line anti-TB drugs)
- Efflux pump inhibitor effect on **baseline MIC** of 1st and 2nd line anti-TB drugs **before** exposure
- RIF exposure at different time points (24hrs, 7 days, 14 days): final **baseline MIC** & EPI effect on anti-TB drugs **after RIF** exposure

Results and Discussion (aim 2)

The drug + EPI combination effect on the baseline MIC before rifampicin exposure

The effect of EPI on baseline MIC before rifampicin exposure



Clinical implications

- Importance of initial drug sensitivity testing
- Provide proof of principle for the synergic effect of drugs and EPI combination in MDR-TB treatment (e.g. TMC207)
- Provide novel insights into the mechanism of drug tolerance/cross-resistance
- This knowledge is critical for the design of novel compounds which will limit the induction of tolerance (e.g. PA-824)

Acknowledgments

- GOD (for being my strength)
- Promoter: Prof TC. Victor
- Co-promoter: Dr. GE. Louw; Prof. RM Warren
- Dr. F. Sirgel, Marianna and Claudia
- TV Lab team & TAS Team
- MRC, US Biomedical Sciences Department

The end… Thank you ☺

Questions are compulsory..

☺☺☺

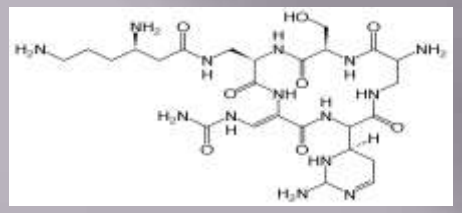
Possible Questions/answers

- Drug structure relation question: Rif derivative of Rifamycin> could be possible for Rifampitine show some similarities. If efflux mechanism is general for structure related e.g.. (interesting for INH Monoresistant strains.)
- E.g. MOXI & OFL> Moxi have unique structure which combines high lipophilicity hydroxyl(methoxy) yet eg OFL have halogen (methyl) bond.
- PZA: prodrug require to be activated into pyrazinoic acid by pyrazinamidaze encoded by pncA @ acidic ph to 5.0 yet in vitro MTB struggle to survive that pH (instead 5.5 & 6 used)..Leonid B. Haiefes;1995.
- Ethionamide: Hiefites et al> could have high MIC from 8-16 (0.2-6)ug/ml. Can produce bactericidal effect(99%) killing conc. close to attainable in vivo esp. when High dosage administered.
- Induction exp: activation of E-Pumps(MIC after will tell) Bactec Growth index /MGIT> GU (hasnt been standardized hnce 7H10 plate use too.)
- EPI exp after Induc: see if addition of EPI (VP) will restore the susceptibility of anti-tb drugs in Rif monoresistant strains

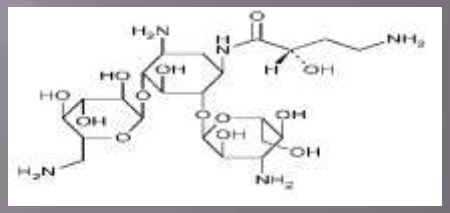
Tolerance:represents a physiological state which enables survival of the bacterium in the presence of antibiotic treatment. **:**prior to removal of the drug, active growth is resumed and bacilli regain full drug susceptibility.

- New drugs include: TMC207 (synergy b/t it & PZA inhibit ATP synthase), SQ109(1ST 2 months of intensive therapy/also to treat MDR-TB), PA-824 (highly active against MDR clinical isolates>suggesting no cross-resistance with current antituberculosis drugs) (Lenaerts, Gruppo et al. 2005) Anastasia ,Petros

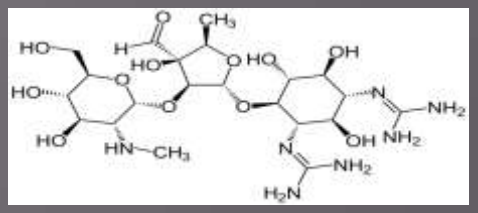
- **MIC according to DST:** Lowest drug concentration that produces complete inhibition of the e bacterial growth in vitro, usually more than 99% of the bacterial population. (Quantitative DST/qualitative).
- **MBC:** lowest concentration that kills at least 99.9% of bacterial population.
- **PAE:** is a persisting suppression of bacterial growth that follows limited exposure to an antimicrobial agent. E.g. an effect that is induced by a pulse exposure rather than by a continuing sub inhibitory concentration.
- **Synergism:** is considered to occur when > 100-fold increase in killing, in comparison with the most active drug alone. Takes place at 24 hrs; yet **antagonism** is defined as > decrease in killing under the same terms. Yet i**ndifference/additivity** is seen when the difference between killing by the drug combination and killing in the presence of the most active single drug is less than tenfold. (6 ref hf book kill-curve method)
- **Early Bactericidal Activity**- bactericidal action against actively multiplying organisms. (drugs in a liquid media: STR,INH&RIF. N EMB & PZA Least bactericidal in vitro.
- **Sterilizing Activity**- as the capacity to kill the persisting organisms: PZA was seen to have a high sterilizing activity in the treatment of affected mice (in vivo) and in TB patients when treated in combination with other 3 drugs INH, STR 7 RIF.
- **CLINICAL EFFICACY (19 REF ON H.F's book)**: PZA Clinical Efficacy of chemotherapy contribution in combination with other drugs is very likely not associated with direct killing but rather with its inhibitory activity against semi-dormant bacteria persisting in an unfavourable acidic environment @ a low ph.
- **Used for the evaluation of two or more drug interactions combined effect.**
- **FIC**= Fractional inhibitory concentration=ratio of MIC in combination/ MIC alone.
- **FBC**=Fractional bactericidal concentration= ratio of MBC in combination/MIC alone



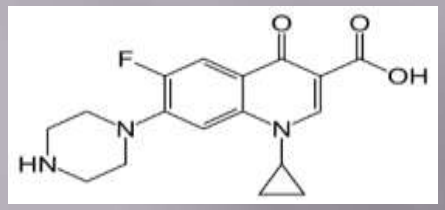
Capreomycin



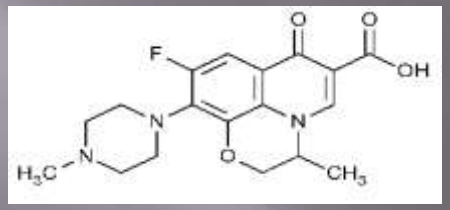
Amikacin



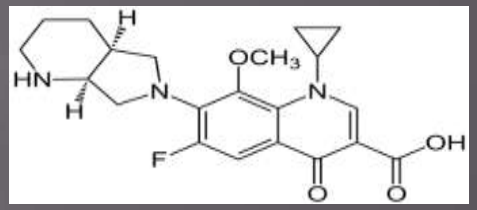
Streptomycin



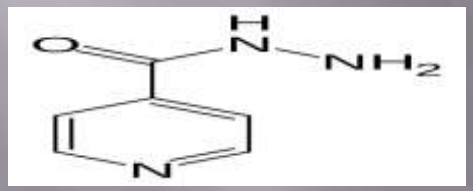
Ciprofloxacin



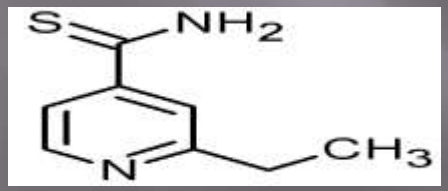
Ofloxacin



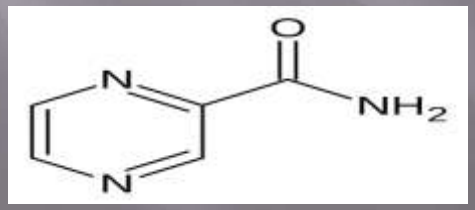
Moxifloxacin



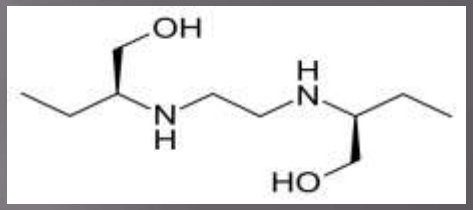
Isoniazid



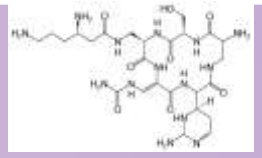
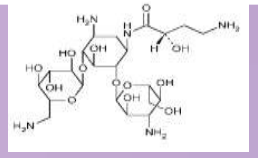
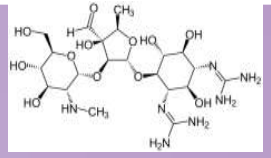
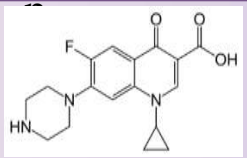
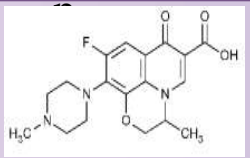
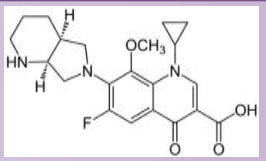
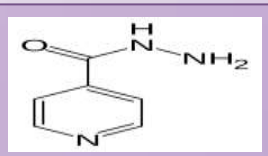
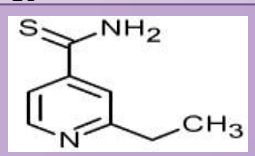
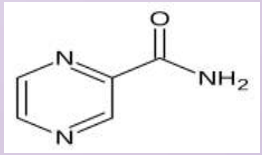
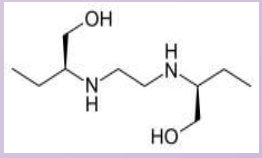
Ethionamide



Pyrazinamide



Ethambutol

Drug Group	Chemical structure		
Group 1 AMINOGLYCOSIDES	 Capreomyci	 Amikaci	 Streptomycin
Group 2 FLUOROQUINOLONES	 Ciprofloxacin	 Ofloxacin	 Moxifloxacin
Group 3 (INH & ETHIO Homolog's)	 Isoniazid	 Ethionamide	
Group 4 (THOSE IN INDIVIDUAL GROUPS)	 Pyrazinamide	 Ethambutol	