THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory

Timely Accurate Diagonostics for a TB-Free Africa

Training on *Mycobacterium tuberculosis* drug susceptibility testing (first and second line LJ DST)

Module 4: Principles of drug resistance to anti-tuberculosis drugs

Venue:

Presenter:

Date:

Introduction

- The module defines the different types of drug resistance, and the mechanism/principles by which drug resistance develops.
- It also provides information on the first and second line anti TB drugs used in the management of TB disease.
- It also provides information on the critical concentration of the different anti TB drugs on the different types of media.

Learning Objective

By the end of the module, participants should be able to:

- Understand the Classical Definitions of drug resistance in TB.
- Attain knowledge on the objectives and rationale of combined anti-tuberculosis therapy
- Understand the Mechanism of Action of 1st & 2nd line anti TB drugs & factors influencing drug resistance development
- Understand Principle behind proportion method for determining drug resistance in *M. tuberculosis*
- Have knowledge on critical concentrations of drugs used for
TB drug susceptibility testing

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Content Outline

- Classical Definitions of drug resistance in TB
- Objectives and rationale of combined anti-tuberculosis therapy
- Mechanism of Action of 1st & 2nd line anti TB drugs & factors influencing drug resistance development
- Principle behind proportion method for determining drug resistance in *M. tuberculosis*
- Critical concentrations of drugs used for TB drug susceptibility testing

Classical definitions of drug resistances

- **Multi drug resistance(MDR);** Resistance to the two major first line drugs **Rifampicin** and **Isoniazid**

- **Extensive drug resistance(XDR):**
  - Resistance to both major first line drugs: **Isoniazid** and **rifampicin**(MDR)
  - Resistance to any **fluroquinolone** and at least one additional Group A drug

Classical definitions of drug resistance

- **Mono resistance**; Resistance to only one anti-TB drug.
- **Poly resistance:** Resistance to more than one anti TB drug
- **Drug resistance among new cases-Previously** referred to as primary resistance.
- **Drug resistance among previously treated patients-** Acquired resistance.
- **Cross resistance**; Resistance between different anti-TB drugs.

Objectives of Anti TB therapy

1. Quickly kill large numbers of rapidly growing bacilli in the infected tissue
   - Cure the patient and increase chances of survival
   - Reduce the infectiousness of patient

2. Prevent the emergence of drug-resistant mutants

3. Sterilization (elimination) of the dormant but still viable bacilli from the infected tissue
   - To avoid therapeutic failure and relapse
   - To reduce the chance of transmission

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Objectives and rationale of combined anti-tuberculosis therapy

- Two drugs introduced for TB therapy in 1940s
  - Streptomycin (SM) and *para*-aminosalicylic acid (PAS)
  - Use of either SM or PAS alone in treatment of TB was initially found to reduce deaths among treated patients

- Soon, it was found that single drug therapy resulted in emergence of drug resistant strains in ~70% of the patients
  - By combining SM + PAS the resistance rate was reduced to 9%

Objectives and rationale of combined anti-tuberculosis therapy

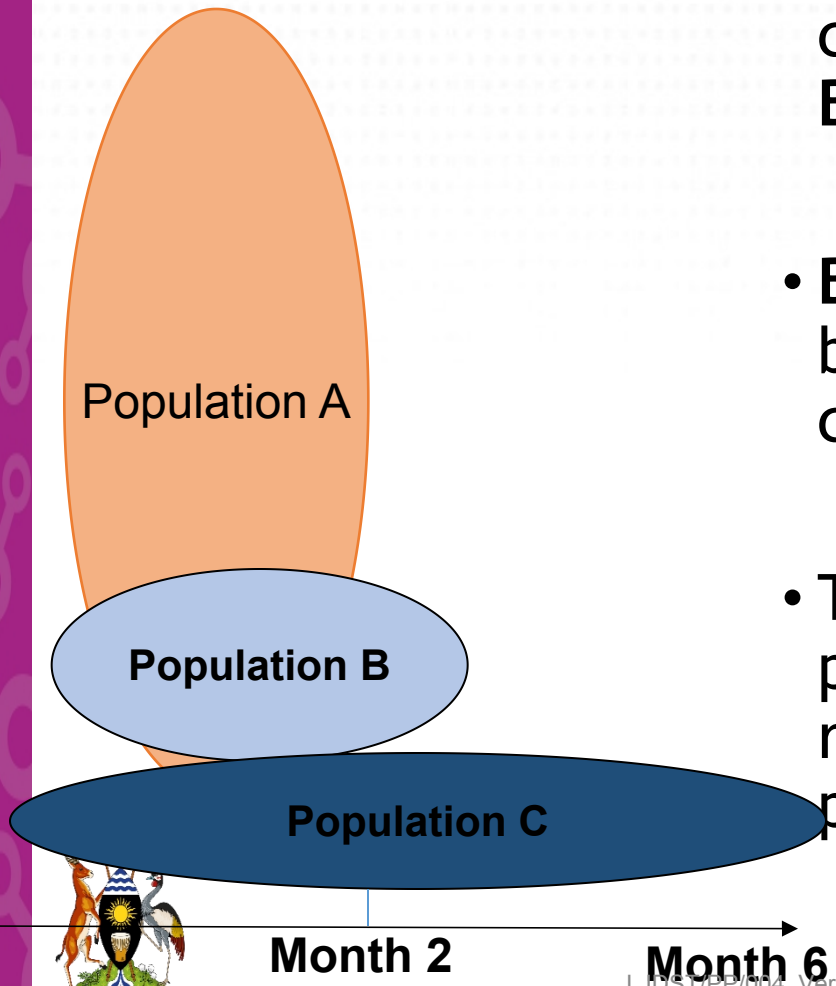
- Isoniazid (INH), more potent drug discovered in 1952
  - Combination therapy – SM + PAS + INH (1950s) proved highly effective in preventing emergence of resistance
  - Still, 18-months of treatment required to ensure adequate cure

- Pyrazinamide (PZA), Ethambutol (EMB) and Rifampin (RMP)
  - PZA (1952), EMB (1962) and RMP (1963) effectively combined with INH for combination therapy

Compartmentalization of *M. tuberculosis* in the infected tissue

- Population A
  - Large number of rapidly dividing bacilli in pulmonary cavities
- Population B
  - Bacilli multiplying less rapidly due to local adverse conditions (most often acidic)
- Population C
  - Dormant but still viable bacilli (often sequestered in granulomas)

Anti TB drugs Act on different populations



- Drugs that kill Population A are considered to have rapid **Bactericidal Activity**
- **Bactericidal Activity** is measured by rapidity of sputum and culture conversion (from + to -)
- These drugs are most effective in preventing the emergence of drug resistant cells that arise in the large populations

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Sterilizing agents

- Drugs that are more effective against Populations B and C are regarded as **Sterilizing** agents.
- The potency of **Sterilizing** activity is reflected by a high cure rate with limited relapses in patients completing therapy

CURRENT FIRST LINE ANTI-TB THERAPY

- Initial two months with Rifampicin, Isoniazid and Pyrazinamide and Ethambutol(RHZE) (Intensive phase)
- Another four months with RMP and INH(RH) (Continuation phase)
- This regimen takes 6months and it;
  - Combines antibacterial activity
  - Inhibits development of resistance
  - Eliminates persisting organisms

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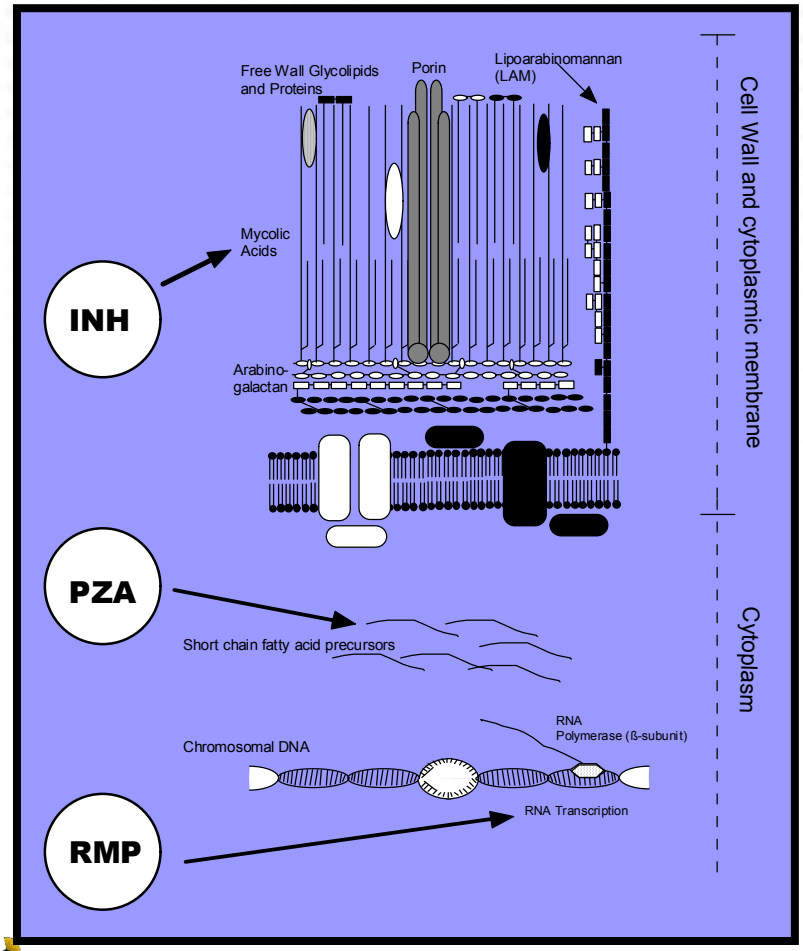
	2nd Line MDR TB regimen (2018)
GROUP A	Levofloxacin Moxifloxacin Bedaquiline Linezolid
GROUP B	Cyclocerine Clofazimine Terizidone Terizidone
GROUP C	Ethambutol Delamanid Amikacin (Streptomycin) Imipenem-cilastatin Meropenem Ethionamide Prothionamide *Para*-aminosalicylic acid

Mechanisms of Action: 1st line Drugs



INH

- Pro-drug, must be activated by catalase (*kat*G)
- Primary **Bactericidal** drug
- Blocks the synthesis of cell wall, acts on other targets in cell

RMP

- Most effective anti-TB drug with both **Bactericidal** and **Sterilizing** activities
- Inhibits bacterial RNA transcription by binding to RNA polymerase

PZA

- Excellent **Sterilizing** effect on Population B (in an acidic environment)
- Pro-drug, must be activated by pyrazinamidase
- Interferes with fatty acid synthesis.

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Mechanisms of Action: 1st line Drugs

- Ethambutol: Exerts excellent bactericidal effect against rapidly multiplying organisms.

Mechanisms of Action: 2nd line drugs

- **Cyclic Polypeptides**: Inhibit Protein synthesis by modifying ribosomal structures at the 16S rRNA. Examples include capreomycin.

- **Fluroquinolones;** Bactericidal antibiotics with excellent activity against *M tuberculosis*.
- Inhibit nucleic acid synthesis through interfearing with the action of DNA gyrase and Topoisomerase IV. Examples incude ofloxacin, Levofloxacin, Moxifloxacin and Gatifloxacin.

Mechanisms of Action: 2nd line drugs

- **Aminoglycosides:** High bactericidal effect.

  Bind to the 30S ribosomal RNA affecting polypeptide synthesis which ultimately inhibits translation in protein synthesis. Examples include, Kanamycin amikacin and streptomycin.

Mechanisms of Action: 2nd line drugs

Drug	Mode of Action	Genes implicated in resistance
Bedaquiline Class : diarylquinoline	Inhibiting mitochondrial ATP synthase.	On target *atpE* off target rv0678, *pepQ,Rv1979c*
Clofazimine Class :riminophenazine	Proposed mechanism of action is production of oxygen reactive species	*rv0678, pepQ* *Rv1979c*
Linezolid Class : oxazolidinone	Protein synthesis inhibitor and it acts mainly on the 50S ribosomal subunit	*rrl* *rplC*
Delamanid and Pretomanid Class : nitroimidazoles	Precise gene targets are still unknown All resistance mutations have been found in genes related to the pro-drug activation	*ddn* *fgd1, fbiA, fbiB, and fbiC* *Rv2983* now known and *fbiD*

Factors influencing the development of drug resistance

Mutations resulting into altearation of:

- Hydrophobic cell envelope (permeability barrier),
- Drug efflux systems and drug-modifying enzymes
  Pump toxic substances out of cell and produce enzymes to change

Metabolism of bacilli shifted to dormancy

•Impaired/decreased drug uptake by *M. tuberculosis* cells

Factors influencing the development of drug resistance

- Impaired drug absorption due to underlying host conditions such as HIV/AIDS
- Treatment with inappropriate drugs, combinations or dosages
- Interruption or irregular treatment
- Incomplete treatment
  - duration
  - required number of doses not taken (patient non-compliant)

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Cross resistance among Second Line Injectable

- Isolates that acquire resistance to amikacin essentially always have associated resistance to kanamycin and capreomycin.

- Isolates that acquire resistance to kanamycin show different levels of cross- resistance with amikacin and capreomycin.

Cross resistance among Second Line Injectables

- Isolates that acquire resistance to streptomycin are usually susceptible to kanamycin, amikacin and capreomycin
- Given the variability in cross-resistance reported for the aminoglycosides, it is recommended that all aminoglycosides (including streptomycin) as well as capreomycin be tested for resistance where possible.

Cross-resistance (Fluroquinolones)

- Owing to extensive cross-resistance among the fluroquinolones, Only one of them should be tested in the laboratory.
- Selection of the most appropriate fluoroquinolone for use in both testing and treatment should be based on a representative surveys or surveillance data.

Principle behind proportion method for determining drug resistance in *M. tuberculosis*

- Laboratory assays **compare** the growth of a 1:100 dilution of the TB isolate on media without drug (Growth Control) to the growth of the undiluted suspension on media containing each drug.

- If the undiluted suspension grows faster or more abundantly in the presence of the drug than the Growth Control, the isolate is considered to contain a resistant population of greater than 1%, and is reported as resistant.

Critical concentrations of drugs used for TB drug susceptibility testing

- **Critical concentration** is the concentration of drug that inhibits the growth of wild type strains without appreciably affecting the growth of resistant cells

- The **critical concentrations** of drugs were found to vary dependent upon the media used (especially solid vs liquid)

Critical concentrations of drugs used for TB drug susceptibility testing

Table 1. Critical concentrations (CC) for first-line medicines recommended for the treatment of drug-susceptible TB.

Medicine	Abbreviation	Critical concentrations (µg/ml) for DST by medium			
		Löwenstein–Jensen[a]	Middlebrook 7H10[a]	Middlebrook 7H11[a]	BACTEC MGIT liquid culture[a]
Rifampicin	RIF	40.0	1.0	1.0	1.0[b]
Isoniazid[c]	INH	0.2	0.2	0.2	0.1
Ethambutol[d]	EMB	2.0	5.0	7.5	5.0
Pyrazinamide[e]	PZA	-	-	-	100

Newer drugs such as BDQ, LZD, &CFZ have not been validated for testing on LJ Media. BDQ binds to protein

Table 3. Critical concentrations (CC) and clinical breakpoints (CB) for medicines recommended for the treatment of RR-TB and MDR-TB. (Interim CC are highlighted in red)

Group	Medicine	Abbreviation	Critical concentrations (µg/ml) for DST by medium			
			Löwenstein Jensen[1]	Middlebrook 7H10[1]	Middlebrook 7H11[1]	BACTEC MGIT liquid culture[1]
Group A	Levofloxacin (CC)	LFX[2,3]	**2.0**	1.0	-	1.0
	Moxifloxacin (CC)	MFX[2,3]	**1.0**	0.5	0.5	0.25
	Moxifloxacin (CB)[4]			2.0	-	1.0
	Bedaquiline[5]	BDQ	-	-	**0.25**	**1.0**
	Linezolid[6]	LZD	-	1.0	1.0	1.0
Group B	Clofazimine	CFZ	-	-	-	**1.0**
	Cycloserine TerizidoneTerizidone	CS TZD	- -	- -	- -	- -
Group C	Ethambutol[7]	E	2.0	5.0	7.5	5.0
	Delamanid[8]	DLM	-	-	**0.016**	**0.06**
	Pyrazinamide[9]	PZA	-	-	-	100.0
	Imipenem-cilastatin Meropenem	IMP/CLN MPM	- -	- -	- -	- -
	Amikacin[10] (Or Streptomycin)	AMK (S)	30.0 4.0	2.0 2.0	- 2.0	1.0 1.0
	Ethionamide Prothionamide	ETO PTO	40.0 40.0	5.0 -	10.0 -	5.0 2.5
	Para-aminosalicylic acid	PAS	-	-	-	-

Assesment

- Define MDR, XDR, Mono resistance, poly resistance and cross resistance?
- What are the 3 populations/compartments of *Mtb?*
- List some of the factors that influence the development of drug resistance?

- What is drug critical concentration as applied in DST?

Summary

- Combined anti-tuberculosis therapy is the cornerstone of effective treatment and prevention of drug resistance.
- The first-line anti-tuberculosis drugs are INH, RMP, PZA and sometimes EMB and SM.
- Second line anti tuberculosis diseases include Amikacin, Levofloxacin, Moxifloxacin among others.

Summary

- *M. tuberculosis* may exhibit natural resistance to certain antibiotics and may develop resistance to anti-tuberculosis agents due to spontaneous mutations in genes encoding drug targets or drug-activating enzymes.

- Anatomical, metabolic compartmentalization, mutation rates and increase in the bacterial load in the lesion may also highly influence the emergence of drug resistance.

References

- GLI TB training package http://www.stoptb.org/wg/gli/trainingpackages.asp
- First and Second Line drugs and Drug Resistance

http://dx.doi.org/10.5772/54960

- Technical manual for drug susceptibility testing of medicines used in the treatment of tuberculosis WHO/CDS/TB/2018.24 © World Health Organization 2018

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