THE REPUBLIC OF UGANDA
MINISTRY OF HEALTH

UGANDA Supranational® Reference Laboratory

Timely Accurate Diagonostics for a TB-Free Africa

Training on Tuberculosis Drug and Susceptibility Testing (MGIT DST – Liquid Method)

Module 4: Principles of DST

Date:

Uganda Supranational Reference Laboratory

Overview

1. History of Anti- TB therapy
2. Introduction to TB drugs
3. Common resistance to TB drugs
4. Drug selection for resistant TB
5. Factors influencing development of drug resistance
6. Challenges for TB DST
7. Methods for determining drug resistance
8. Summary
9. References

History of 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%

Introduction of more effective anti TB drugs

Isoniazid (INH), more potent drug,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

Current four-drug TB therapy (first-line, most effective drugs)

Requirements for effective cure

- Treatment with multiple antibiotics
- Long therapy – 6 months

Initial two months with RMP, INH and PZA and either EMB or SM

Another four months with RMP and INH and this regimen

- Combines antibacterial activity
- Inhibits development of resistance
- Eliminates persisting organisms

	2nd Line MDR TB regimen (2018)
GROUP 1	Levofloxacin Moxifloxacin Bedaquiline Linezolid
GROUP 2	Cyclocerine Clofazimine Terizidone
GROUP 3	Ethambutol Delamanid Amikacin (Streptomycin) Imipenem-cilastatin Meropenem Ethionamide Prothionamide *Para*-aminosalicylic acid

Objectives of antituberculosis 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

Compartmentalization of M. TB in the infected tissue

Population A

- Large number of rapidly dividing bacilli in pulmonary cavities

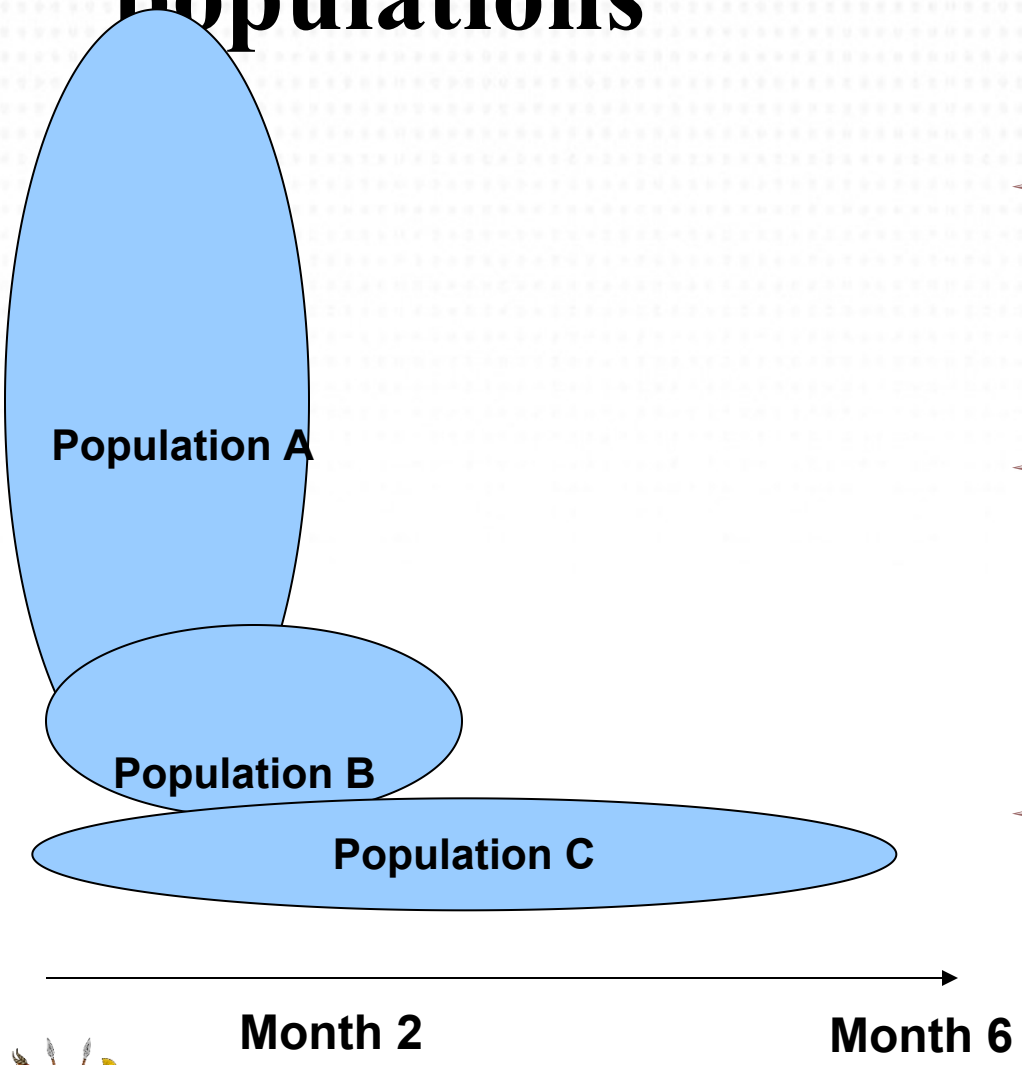
Population B

- 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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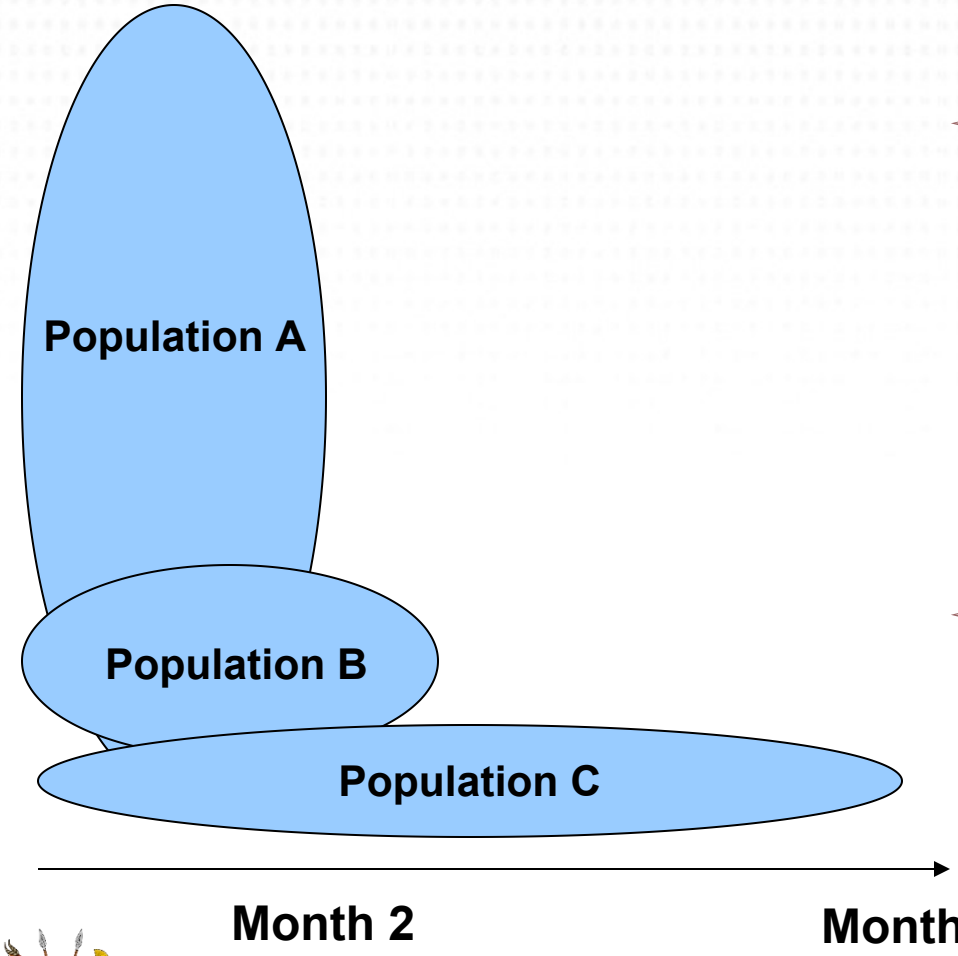
Sterilizing effect



- 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

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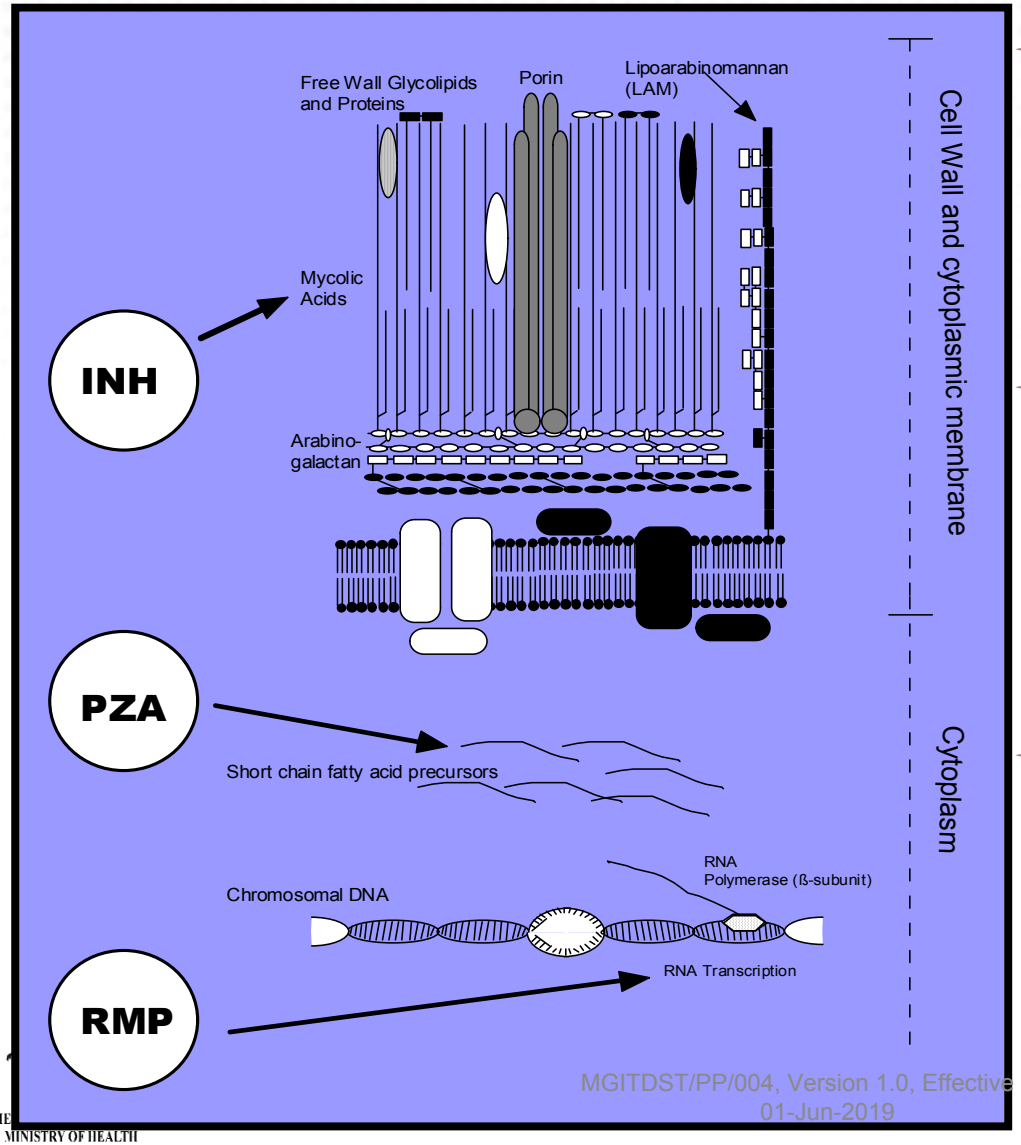
First-line antituberculosis drugs and their mechanisms of action



- 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

Natural drug resistance in the MTB complex

MTB complex members

- Most common - *M. tuberculosis, M. bovis*
- Vaccine strain - *M. bovis* BCG
- Rarely seen - *M. africanum, M. canettii, M. microti, M. caprae, M. pinnipedii*

Natural resistance to antibiotics

- Hydrophobic cell envelope (permeability barrier),
- Drug efflux systems and drug-modifying enzymes
  - Pump toxic substances out of cell and produce enzymes to change the drug structure
- PZA resistance in *M. canettii, M.bovis* and BCG (other members are usually susceptible to PZA)

Common types of resistance to first-line drugs – WHO classifications

Drug resistance in newly identified patients

- Previously defined as *primary drug resistance*

Drug resistance in previously treated patients

- Previously defined as *acquired drug resistance*

Cross-resistance between different drugs

- Drugs are chemically related (similar structure)
- Drugs have the same or similar target within the bacterial cell
- Rifamycins, aminoglycosides, fluoroquinolones

Resistance in TB to more than one drug

Polyresistance

- Strains of TB resistant to more than one antituberculosis drug

Multi-drug resistant TB (MDR-TB)

- Strains of TB resistant at least to INH and RMP
- Results in treatment failure and fatal outcomes more often than with resistance to other drugs

Extensively drug resistant TB (XDR-TB)

- MDR-TB also resistant to any of the fluoroquinolones and to at least one injectable drug
- Often untreatable

Selection of drug resistant mutants in TB

Spontaneous mutations occur in the DNA of all cells

- Mutations can change the structure of a protein that is a drug target
- Protein still functions, but is no longer inactivated by the drug
- Thus, TB can grow in the presence of the drug

Resistance is linked to large bacterial populations

- Mutants resistant to any drug occur on average once in every 100 million (10^8) cells
- In TB in the lung, cavities often contain 10^7–10^9 organisms
- Non-cavitary lesions contain about 10^3–10^4 organisms
- By using two antibiotics, chances for both targets to be mutated is extremely small ($10^{-8} \times 10^{-8} = 10^{-16}$)
- Monotherapy led to selection of drug-resistant populations in cavitary disease more often than in cases with non-cavitary lesions
- This is the rationale for treatment regimens with more than one drug

Other factors influencing the dev't of drug resistance

Metabolism of bacilli shifted to dormancy

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

Penetration of drugs to various body sites

- Suboptimal concentration of drugs at some sites

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)

Challenges for TB drug susceptibility testing

- To determine the critical concentrations of drugs necessary to eliminate the growth of susceptible (wild type) strains of *M. tuberculosis* in susceptibility testing

- To determine what proportion of cells in a population of *M. tuberculosis* would need to be resistant to a drug in order for that strain to be interpreted 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)

Determination of clinically significant proportion of resistant bacteria

Clinically significant proportion

- ***In vitro*** growth of resistant cells in the presence of the critical concentration of the drug that is equal to or greater than 1% of the growth of the total population in the absence of the drug

The presence of 1% resistant cells

- Represents a significant increase in resistance,
- Laboratory assays are based on this assumption

The 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.

Summary (1)

1. Combined antituberculosis therapy is the cornerstone of effective treatment and prevention of drug resistance.

2. The first-line antituberculosis drugs are INH, RMP, PZA and sometimes EMB and SM.

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

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

Summary (2)

5. WHO recommends use of the phrase “drug resistance among new cases” instead of primary resistance, and “drug resistance among previously treated patients” instead of acquired resistance.

6. Critical concentration is the amount of drug in the medium that inhibits the growth of susceptible organisms but not that of the resistant mutants

7. The clinically significant proportion of resistant mutants (determined to be greater than 1% of the population) indicates the magnitude of drug resistant cells that either predict or reflect therapeutic failure.

References

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Acknowledgement