Mechanism of action of antimicrobial agents

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What is an Antibiotic?

- An antibiotic is a selective toxin
- It has been chosen so that it will kill the desired bacteria, but not the cells in your body. Each different type of antibiotic affects different bacteria in different ways.
- For example, an antibiotic might inhibit a bacteria's ability to turn glucose into energy, or the bacteria's ability to construct its cell wall. Therefore the bacteria dies instead of reproducing.

Selective toxicity

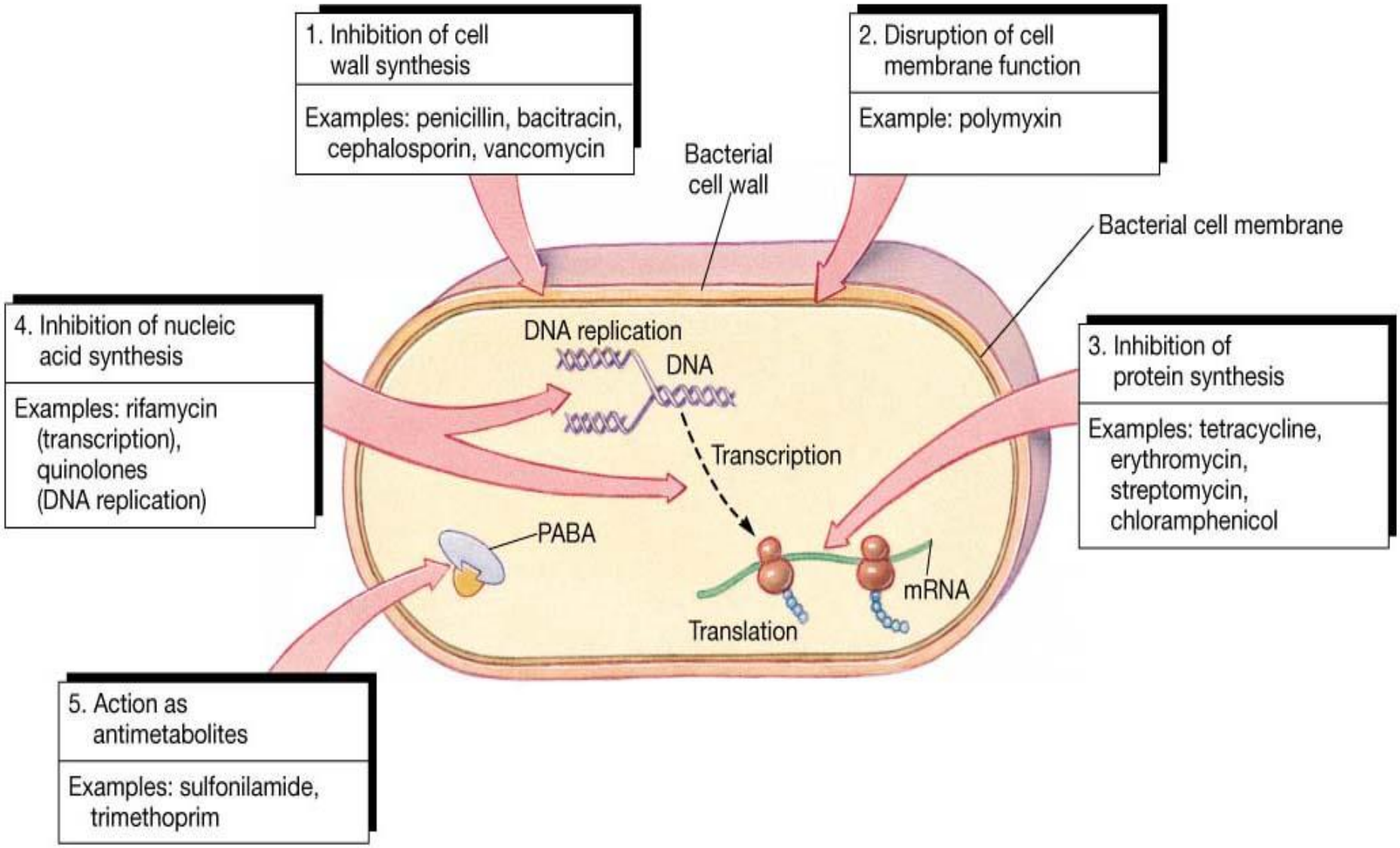
- The aim of antimicrobial therapy is to kill or inhibit the infecting organism without damaging the host; this is known as selective toxicity. This is commonly accomplished through the use of antimicrobial drugs

Bacteriostatic vs. Bactericidal

- Antibiotics differ by mode of action
- Bacteriostatic compounds inhibit the growth of bacteria
  - Holds invaders in check; host immune system does the killing
- Bactericidal compounds directly kill the bacteria
- Location and severity of infection affect choice of antibiotic
  - E.g. CNS infection calls for bactericidal treatment.

Antibacterial Agents

- A. Inhibitors of cell wall synthesis
  1. Penicillins
  2. Cephalosporins
  3. Other antibacterial agents that act on cell walls
- B. Disrupters of cell membranes
  1. Polymyxins
  2. Tyrocidins
- C. Inhibitors of protein synthesis
  1. Aminoglycosides
  2. Tetracyclines
  3. Chloramphenicol
  4. Other antibacterial agents that affect protein synthesis
     a. Macrolides
     b. Lincosamides
- D. Inhibitors of nucleic acid synthesis
  1. Rifampin
  2. Quinolones
- E. Antimetabolites and other antibacterial agents
  1. Sulfonamides
  2. Isoniazid
  3. Ethambutol
  4. Nitrofurans


1. Inhibition of cell wall synthesis
Examples: penicillin, bacitracin, cephalosporin, vancomycin
2. Disruption of cell membrane function
Example: polymyxin
Bacterial cell wall
Bacterial cell membrane
4. Inhibition of nucleic acid synthesis
Examples: rifamycin (transcription), quinolones (DNA replication)
DNA replication
DNA
Transcription
3. Inhibition of protein synthesis
Examples: tetracycline, erythromycin, streptomycin, chloramphenicol
PABA
mRNA
Translation
5. Action as antimetabolites
Examples: sulfonilamide, trimethoprim

1-Inhibition of cell wall synthesis

- beta-lactam containing antibiotics inhibit transpeptidase; bacteria cannot synthesize reinforced cell wall and they lyse when they try to grow
- Vancomycin and cyclo-Ser inhibit specific binding of Ala's in crossbridges to transpeptidase in many gram+ bacteria
- Bacitracin inhibits secretion of NAG and NAM subunits
- All of these only kill growing bacteria

D-Cycloserine

There are three stages of peptidoglycan biosynthesis The first occurs in the cytoplasm where the precursors are synthesized. The formation and assembly of a D-alanyl-D-alanine dipeptide is the site of action of D-cycloserine.

second stage of the biosynthetic pathway in which the disaccharide pentapeptide is transported across the membrane on the lipid carrier to be inserted into the cell wall at a growing point.

It is in the third and final stage of the pathway that the glycopeptide antibiotics act. Here the disaccharide pentapeptide is first incorporated into the expanding cell wall.

this step is effectively blocked by the glycopeptides.

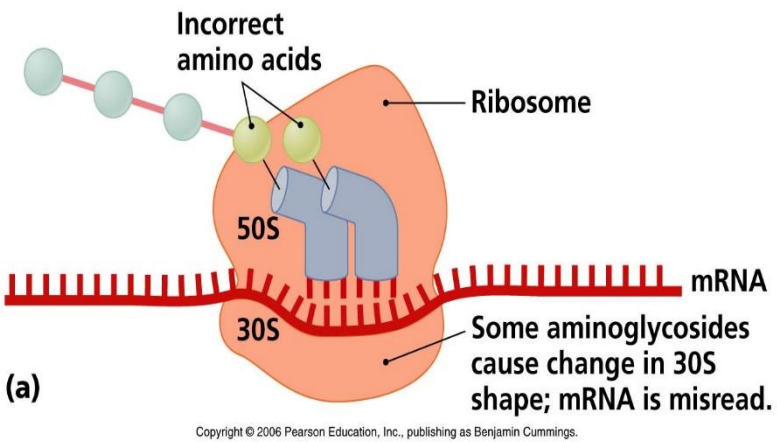
2- Inhibition of protein synthesis

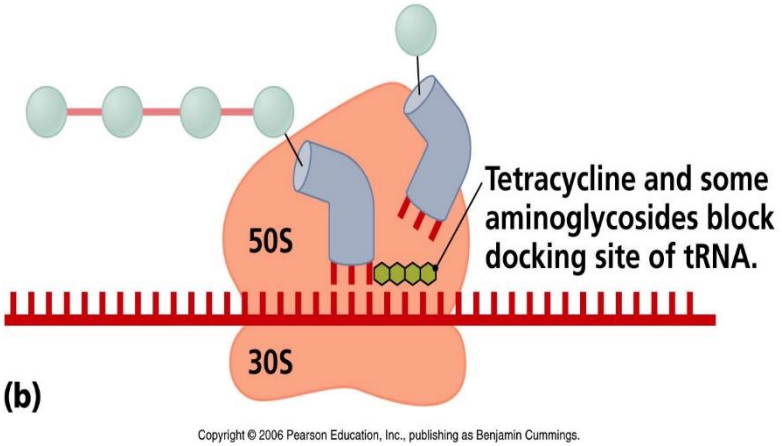
- **Aminoglycosides** (bactericidal): streptomycin, kanamycin, gentamicin, tobramycin, amikacin, netilmicin, neomycin
- **Macrolides**
- **Chloramphenicol, Lincomycin, Clindamycin (bacteriostatic)**

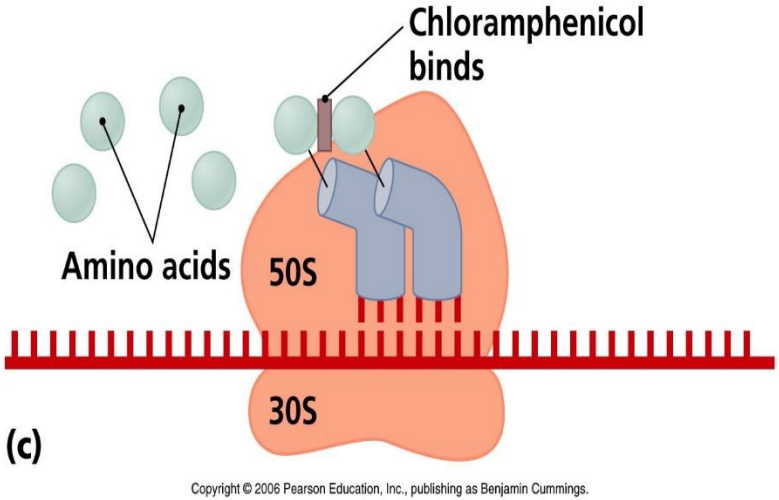
Protein synthesis inhibitors

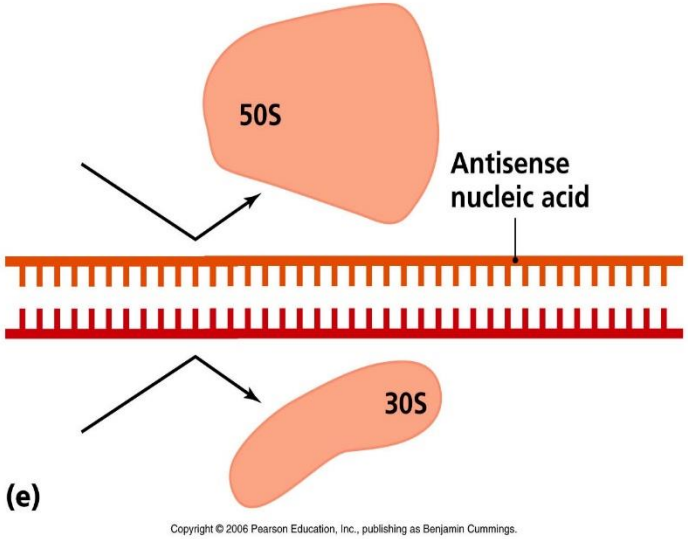
- Need to affect bacteria, not mitochondria
- Aminoglycosides (streptomycin, gentamicin) change shape of 30S ribosome subunit
- Tetracycline blocks access to A site of 30S subunit
- Chloramphenicol block peptide bond formation from 50S subunit
- Macrolides (erythromycin) block 50S subunit action
- Antisense NAs bind to beginning of mRNA and block translation

Protein synthesis inhibitors









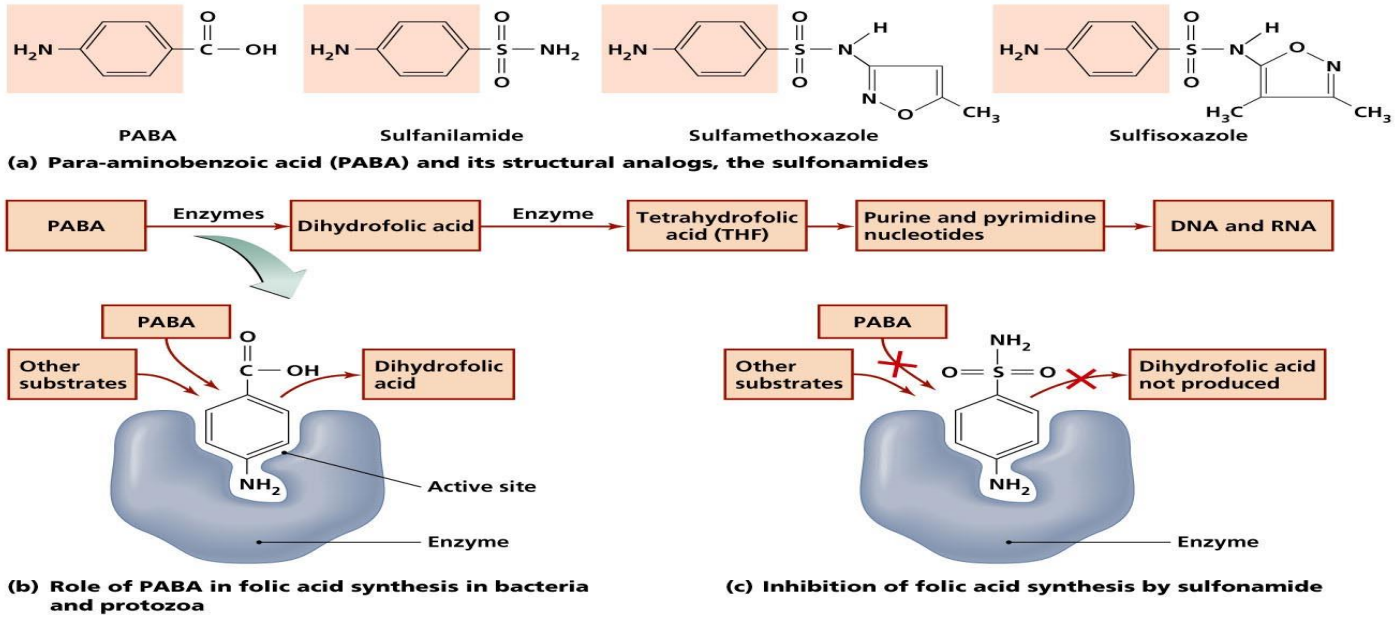
Ribosomes: site of protein synthesis

- Prokaryotic ribosomes are 70S;
  - Large subunit: 50 S
    - 33 polypeptides, 5S RNA, 23 S RNA
  - Small subunit: 30 S
    - 21 polypeptides, 16S RNA
- Eukaryotic are 80S

  Large subunit: 60 S
    - 50 polypeptides, 5S, 5.8S, and 28S RNA
  - Small subunit: 40S
    - 33 polypeptides, 18S RNA

Differences in structure between prokaryotic and eukaryotic ribosomes make antibiotics that target protein synthesis fairly selectively toxic against bacteria.

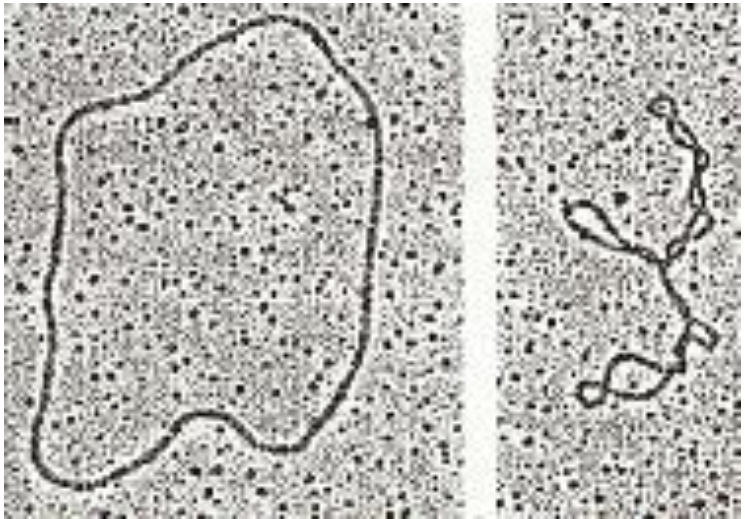
3- Metabolic inhibitors



- Sulfonamides (sulfanilamide) are structural analogs of PABA, a molecule crucial for Nucleic acid synthesis
- humans do not synthesize dihydropteroic acid from PABA
- Trimethoprim interferes in next step

4-Interference with nucleic acid synthesis

- Bacterial DNA is negatively supercoiled
  - Supercoiling is maintained by gyrase, a type II topoisomerase.
  - Inhibition of gyrase and type IV topoisomerase interferes with DNA replication, causes cell death
  - Eukaryotic topoisomerases differ in structure



Quinolones (bactericidal)

nalidixic acid, **ciprofloxacin**, ofloxacin, norfloxacin, levofloxacin, lomefloxacin, sparfloxacin

- **Mode of action -** These antimicrobials bind to the A subunit of DNA gyrase (topoisomerase) and prevent supercoiling of DNA, thereby inhibiting DNA synthesis.

- **Spectrum of activity -** Gram-positive cocci and urinary tract infections

5- Cell membranes as targets

- Bacterial cell membranes are essentially the same in structure as those of eukaryotes
  - Antibiotics also affect Gram neg. cell walls, ie. Outer membrane together with cell membrane
  - Anti-membrane drugs are less selectively toxic than other antibiotics.
  - Many antifungal drugs (Polyenes as Amphotericin B, Nystatin) make use of cell membrane differences.

Cell membrane disruptors

- Amphotericin B binds to ergosterol of cell membranes of fungi, causing lysis of cell
- Azoles (fluconazole) and allyamines (turbinafine) block ergosterol synthesis
- Polymixin disrupts bacterial cell membranes, but is toxic to people

Table 8.1 Target sites for antimicrobial action

Target	Antibioticst	Mechanism of action	Basis of selective toxicity
Bacterial cell wall	j3-Lactams	Inhibit peptidoglycan synthesis	None in mammalian cells
	Glycopeptides	Inhibit peptidoglycan synthesis	None in mammalian cells
	Cycloserine	Inhibits peptidoglycan synthesis	None in mammalian cells
	Isoniazid*	Inhibits mycolic acid synthesis	None in mammalian cells
	Ethambutol*	Inhibits arabinogalactan synthesis	None in mammalian cells
Bacterial ribosome function	Aminoglycosides	Distort 30S ribosomal subunit	No action on 40S subunit
	Tetracyclines	Block 30S ribosomal subunit	Excluded by mammalian cells
	Chloramphenicol	Inhibits peptidyl transferase	No action on mammalian equivalent
	Macrolides, azalides	Block translocation	No action on mammalian equivalent
	Fusidic acid	Inhibits elongation factor	Excluded by mammalian cells
	Mupirocin	Inhibits isoleucyl-tRNA synthesis	No action on mammalian equivalent
Chromosome function	Quinolones	Inhibit DNA gyrase	No action on mammalian equivalent
	Metronidazole (also**)	DNA strand breakage	Requires anaerobic conditions not present in mammalian cells
	Nitrofurantoin	DNA strand breakage	
	Rifampicin (also*)	Inhibits RNA polymerase	No action on mammalian equivalent
	5-Fluorocytosine***	Inhibits DNA synthesis	Converted to active form in fungi
Folate metabolism	Sulphonamides (also**)	Inhibit folate synthesis	Not present in mammalian cells
	Trimethoprim	Inhibits dihydrofolate reductase	Mammalian enzyme not inhibited
	Pyrimethamine**	Inhibits dihydrofolate reductase	Mammalian enzyme not inhibited
	Trimetrexate**/***	Inhibits dihydrofolate reductase	Toxicity overcome with leucovorin
Cytoplasmic membrane	Polymyxins	Disrupt bacterial membranes	Bind to LPS and phospholipids
	Polyenes***	Disrupt fungal membranes	Bind preferentially to ergosterol
	Imidazoles and triazoles***	Inhibit ergosterol synthesis	Pathway not in mammalian cells
	Naftidine***	Inhibits ergosterol synthesis	Pathway not in mammalian cells

t All antibacterial except: *antimycobacterial agent; **antiprotozoal agent; ***antifungal agent.

LPS, lipopolysaccharide.

Spectrum

- When specific testing is not done or delayed, antibiotic with a broad spectrum is administered
  - Broad spectrum antibiotics can penetrate Gram – outer membranes, resist inactivation, etc.
  - Shotgun: better chance of inhibiting pathogen
- Death of normal microbiota results in overgrowth of resistant bacteria (endogenous infection; "superinfection") or allows invasion by outside opportunists.

Anti M.TUBERCUIOSIS

First line Drugs	Mechanism of action	Main adverse effects[1]	Dose mg/kg/Day (range) [Max][2]
Isoniazid (H)	Bactericidal-inhibits cell wall synthesis, most potent bactericidal, rapidly kills actively metabolizing bacilli	Hepatitis Peripheral neuropathy	10 (10 – 15) [300 mg]
Rifampicin (R)	Bactericidal-inhibits RNA synthesis, kills extra-cellular and slow growing bacilli.	Hepatitis, orange color secretions drug-drug interactions	15 (10 – 20) [600mg]
Pyrazinamide (Z)	Disrupts energy metabolism- kills bacilli within granulomas	Hepatitis Arthralgia	35 (30 – 40) [2000mg]
Ethambutol (E)	Bacteriostatic – inhibits cell wall synthesis, prevents drug resistance	Visual disturbance [2]	20 (15 – 25) [1200mg]