Microbiology

Lecture No:......4.............

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Sheet ☐ Slide ■

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Antimicrobial drugs

By

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Introduction

- The use of antimicrobial drugs is successfully control the majority of bacterial, parasitical, fungal infections which affect human and animals.

- **Sulfonamide** 1934, **Penicillin G** 1941 obtained from **Penicillium notatum**..

- Aminoglycosides (Streptomycin, Kanamycin)1946.. Obtained from soil bacteria Actinomycetes group.

- At present about 100 antimicrobial drugs of different classes are available for use in humans.

- Clinically effective antimicrobial agents should exhibit selective toxicity toward the bacterium not the host.. Few Side Effects.. Good pharmacokinetics

General Antimicrobial Effects

- Drugs kill only actively growing microorganisms are termed **bactericidal**

  Penicillins, Aminoglycosides

- Drugs that only inhibit the growth of microorganisms are termed **bacteriostatic**..

  Sulfonamides, Chloramphenicol, Tetracyclines

- The decision to use a bactericidal / bacteriostatic drug to treat infection depends entirely upon the type & body site of infection, patients age, kidney–Liver functions.. acute or chronic infection.

- Ultimate elimination of the organisms is dependent upon host immune defense..phagocytic activity & specific antibodies
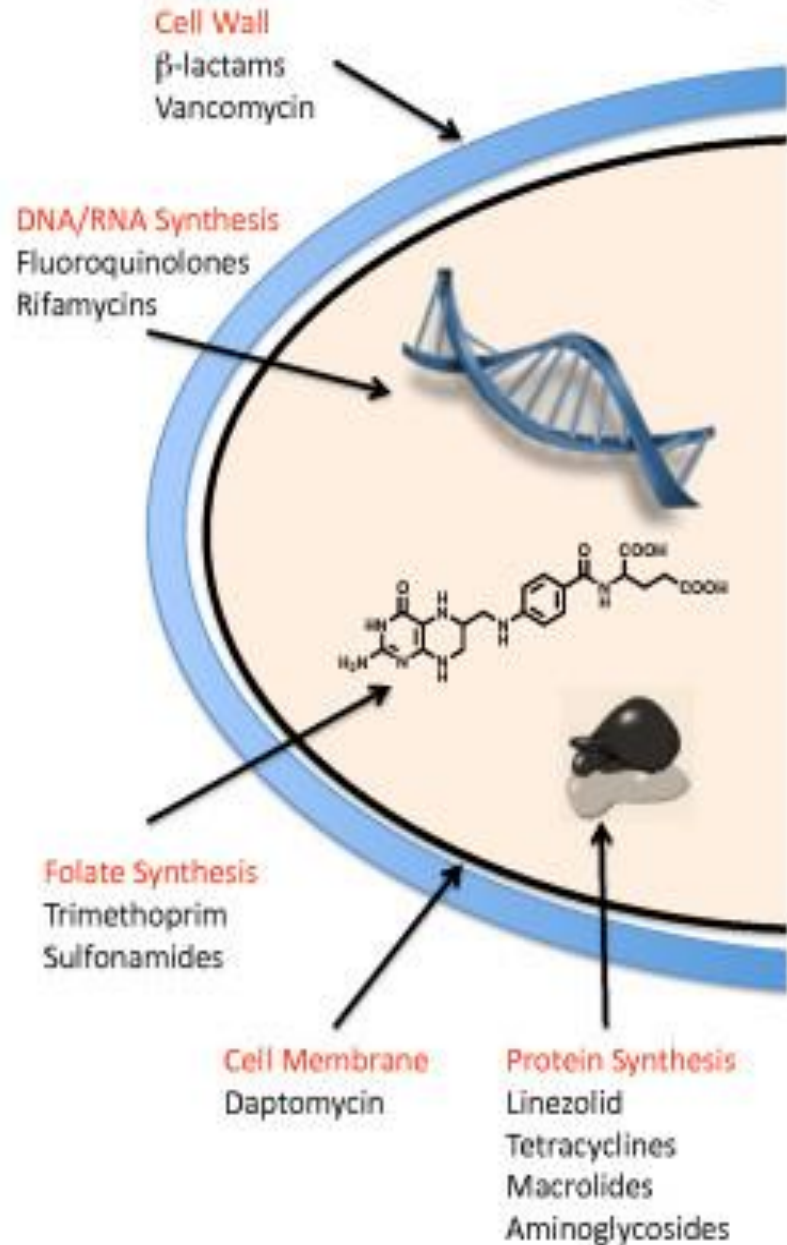
Action of Antimicrobial Drugs on Bacteria

Antimicrobials are classified: Range of activity/spectrum..

- **Narrow** : Vancomycin, Penicillin (G+ve), Antimycobacterial drugs
  Moderate: (G-ve/G+ve) Ampicillin , Amoxicillin,

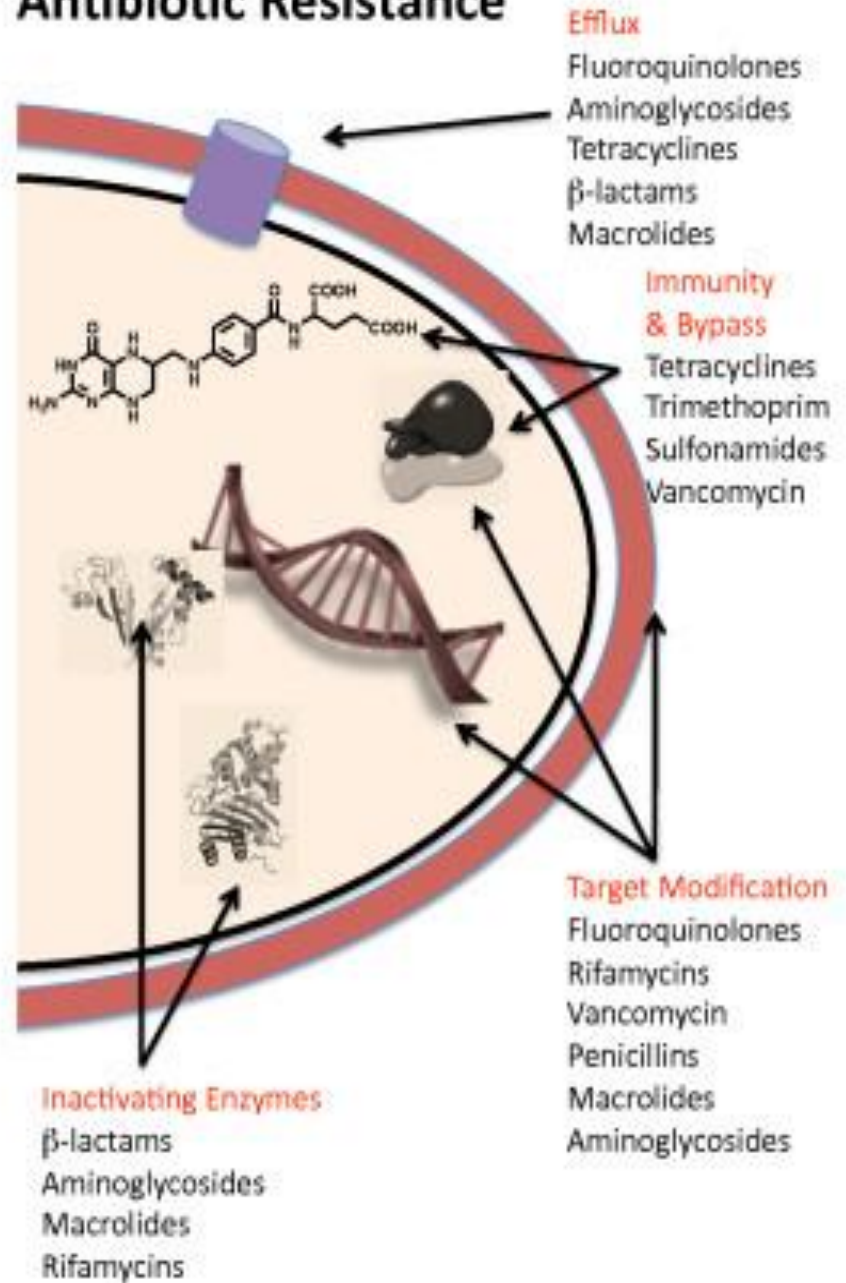- **Broad spectrum:** (G-ve/G+ve) Tetracylines, Chloramphenicol
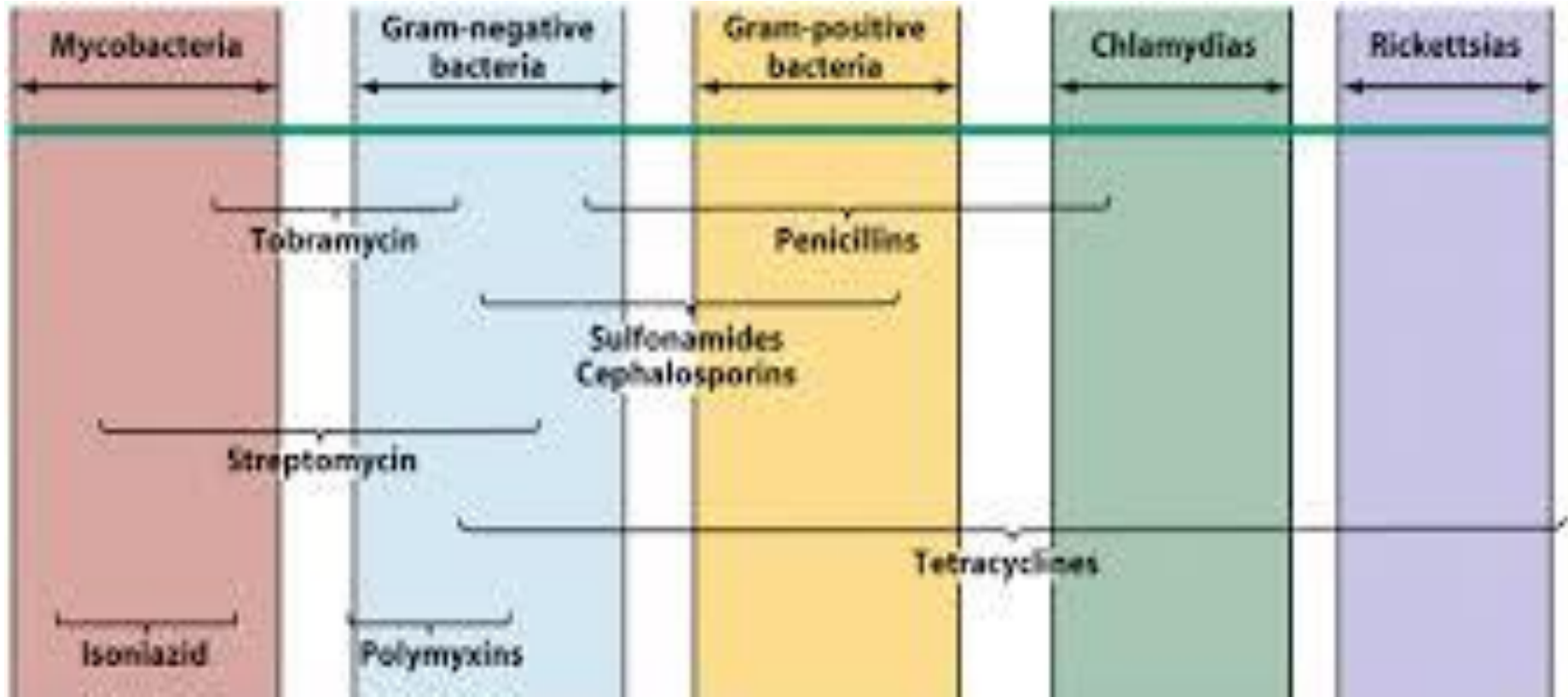
- Antimicrobials affect specific or various **bacterial cellular targets**:
- cell wall,
- plasma membrane,
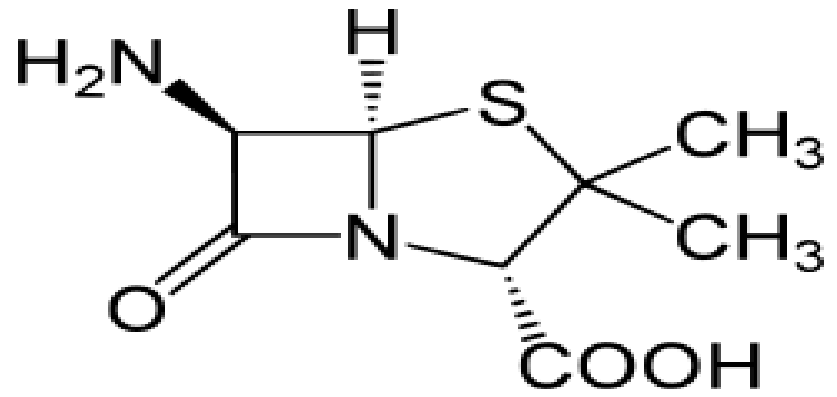- nucleic acids,
- proteins synthesis.

Antibiotic Targets

Cell Wall
β-lactams
Vancomycin

DNA/RNA Synthesis
Fluoroquinolones
Rifamycins

Folate Synthesis
Trimethoprim
Sulfonamides

Cell Membrane
Daptomycin

Protein Synthesis
Linezolid
Tetracyclines
Macrolides
Aminoglycosides

Antibiotic Resistance

Efflux
Fluoroquinolones
Aminoglycosides
Tetracyclines
β-lactams
Macrolides

Immunity & Bypass
Tetracyclines
Trimethoprim
Sulfonamides
Vancomycin

Target Modification
Fluoroquinolones
Rifamycins
Vancomycin
Penicillins
Macrolides
Aminoglycosides

Inactivating Enzymes
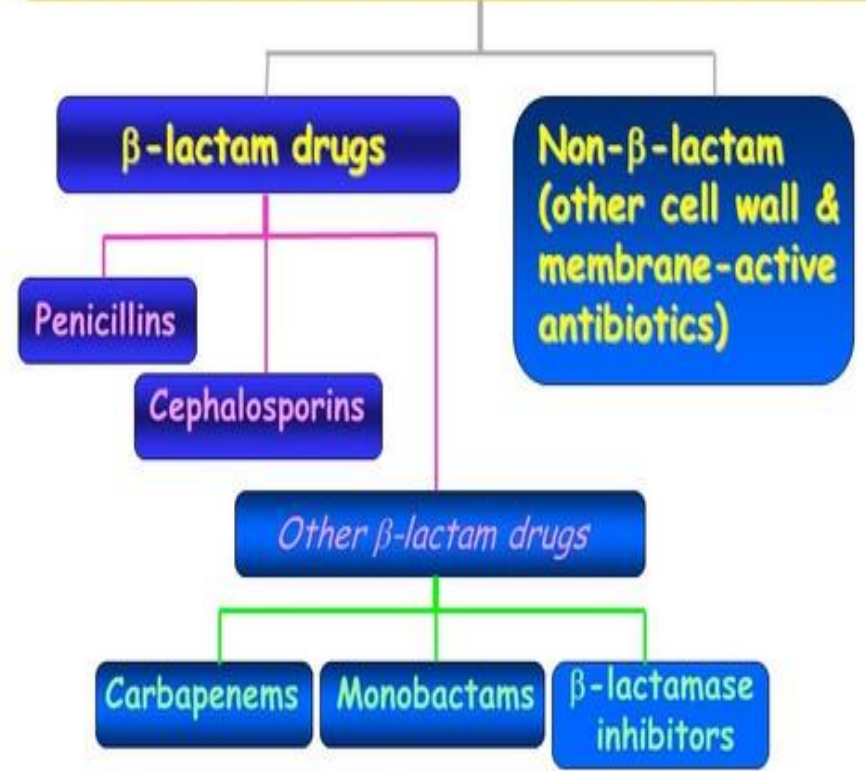β-lactams
Aminoglycosides
Macrolides
Rifamycins

1- **Inhibition Cell Wall Synthesis:**

- Group of 6-Amino penicillanic acid include all Beta-Lactam drugs

- Bactericidal..

- They differs only by the presence of an **amino /carboxyl group**.. These Help the drug penetrate the outer membrane of gram-negative bacteria.
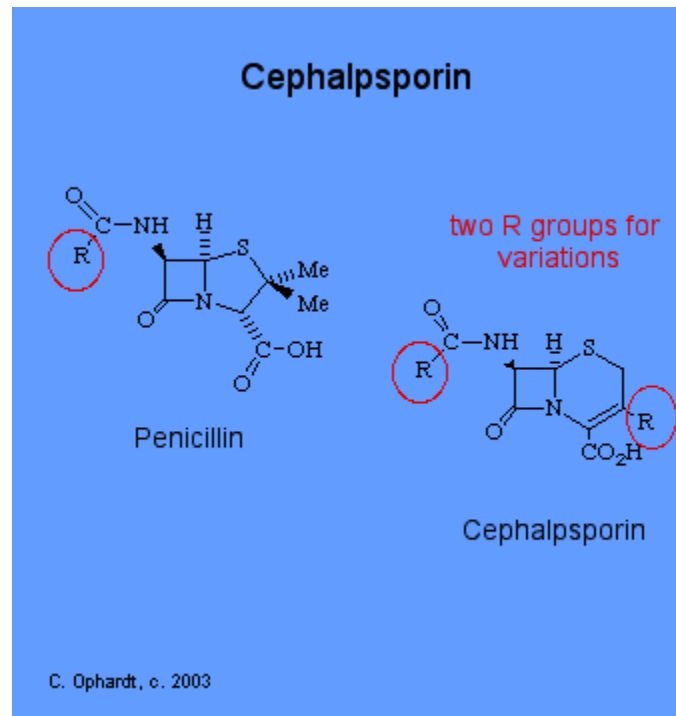

Cell Wall and Membrane-Active Antibiotics

- β-lactam drugs
  - Penicillins
  - Cephalosporins
  - Other β-lactam drugs
    - Carbapenems
    - Monobactams
    - β-lactamase inhibitors
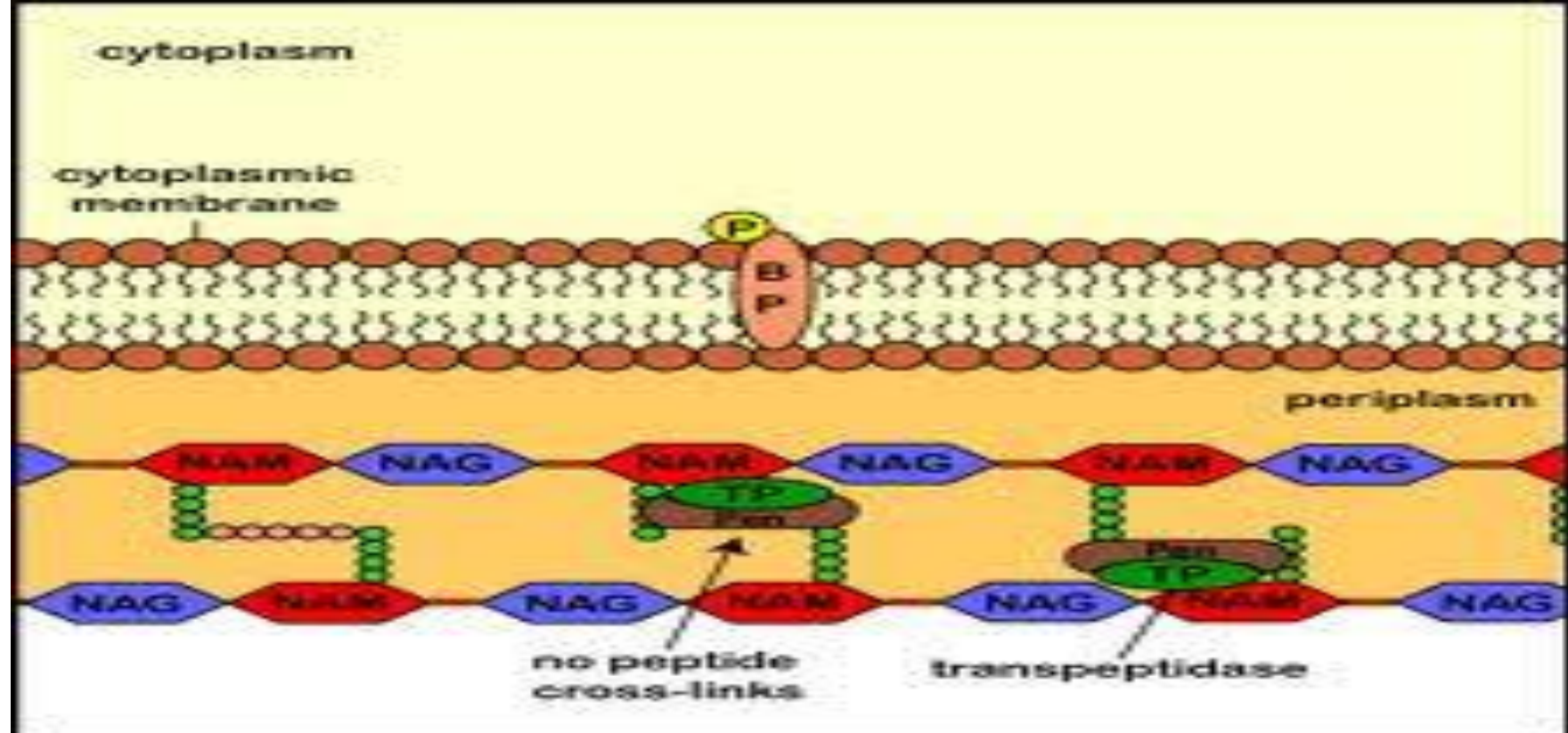- Non-β-lactam (other cell wall & membrane-active antibiotics)
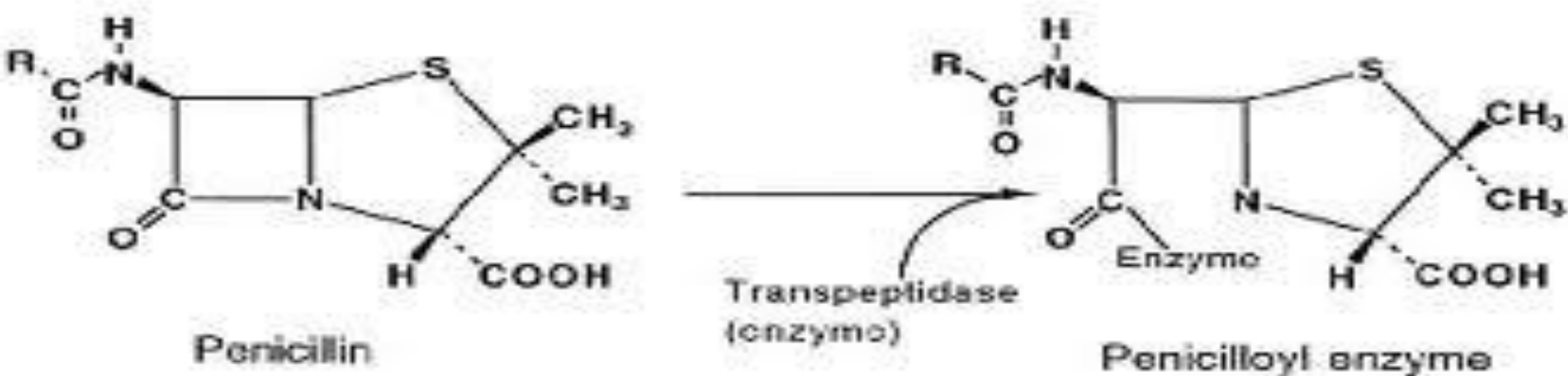
Beta-Lactam Structures
Benzylpenicillin (5-Thazolidine Ring)

Cephalosporins (6-Dihydrothiazine Ring)

Mechanism of Penicillin Inhibition of Transpeptidase Enzyme

Inhibition Cell Wall-1

- **All Beta-Lactam Drugs**

  Attached to Penicillin Binding Proteins (PBPs)/ found as both membrane-bound and cytoplasmic proteins.. Necessary to produce final stages of **peptidoglycan.**

- These drugs inhibit **transpeptidases** that cross-linking of growing peptidoglycan.. Stop cell wall synthesis & activation cell autolysins.

- **1- Narrow spectrum;** Penicillin G, V

- Affects mainly G+ve aerobic & anaerobic bacteria

- Less G-ve facultative anaerobic.

- *Streptococci, Staphylococci, Bacteroides.*

- **2- Moderate spectrum;** Ampicillin, Amoxacillin

  G+ve/G-ve. . All B-lactam drugs can become susceptible to Penicillinases /ß-Lactamases actions.

- **3- Penicillinase-R drugs:**
- **Oxacillin, flucloxacillin,Methicillin** (1970s) used only against Staph-R to Penicillins-Ampicillin..
- Methicillin-R *Staph. aureus* (MRSA) in Jordan up 70%, *mecA gene*.. Worldwide distribution.. Serious Infections.
- **Amoxacillin+Clavulinic Acid** (B-lactamase inhibitor) Broad Spectrum.. Against Penicillinase-R
- **Carbencillin, Piperacillin (**1970s) Carboxypenicillins used mainly against G-ve *Pseudomonas spp.*
- **Monobactam: β-lactam ring is alone**.. Aztreonam.. Effective only against G-ve R-Enteric bacteria
- **Carbapenem:** Imipenem & Meropenem (2000) Broad Spectrum, Highly resistance to most penicillinases ..including **Extended beta-lactamases**.. Serious Nosocomial Infection, Enteric bacilli., *P. aeruginosa, Acinetobacter* spp.
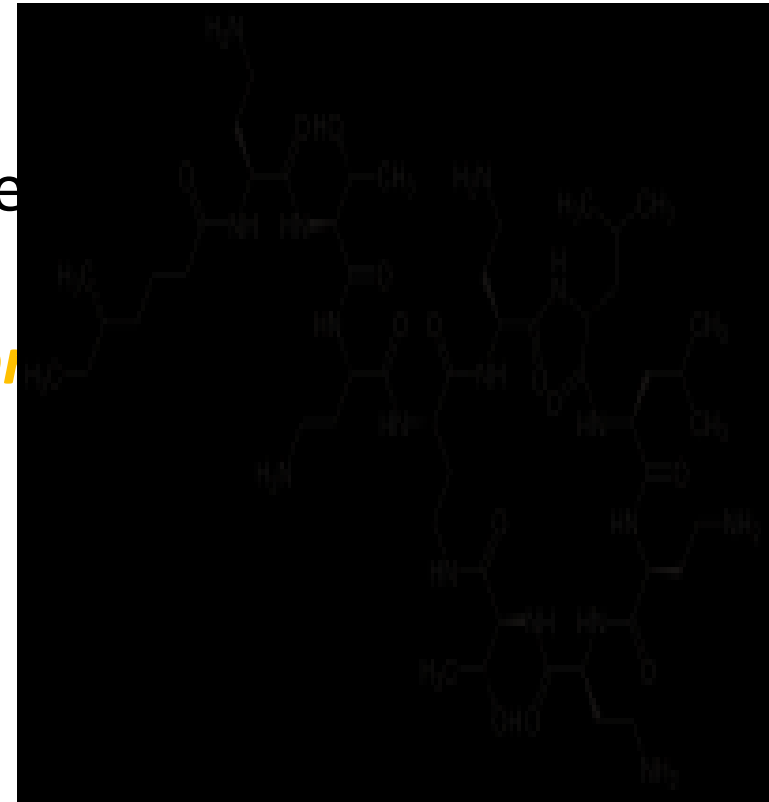
Inhibition Cell Wall-3

- **4- Cephalosporins**: 4 Generations..1965-1990s..Oral, IV, IM.
- 1th (1960) *Cephalexin*, *Cephradine*, <u>Broad spectrum</u>..
- 2th (70s) *Cefoxitin*, *Cefuroxime*, <u>Broad spectrum</u>..
- 3th (80s) *Ceftriaxone*, *Cefotaxime*.. <u>mainly G-ve Enteric bacteria</u>..but effective against some G+Ve

  bacteria *Streptococcus pneumoniae*
- 4th (90s) *Cefepime*.. Mainly G-ve Enteric bacteria
- UTI, RTI, Intestinal, Blood sepsis, CSF infections..
- Not used against anaerobes
- All increased resistance Enterococcus group (*E. fecalis, E.faecium*) in human intestinal.

Inhibition Cell Wall-4

- **<u>Resistance Development</u> :**
- All G-ve enteric bacteria especially

   - E.coli, Klebsiella/Enterobacter spp., *P.aeruginosa &*
   Acinetobacter spp.. Develop rapidly resistance by **mutation**
   & Plasmid transfer <u>ß-lactamases genes</u>..

   - <u>Extended ß-lactamases</u> (> 60 types)..

   - Altered Penicillin Binding Proteins.. Inactive Penicillin
   drugs

   - Spread mostly in hospitalized patients.

- **<u>Side Effects:</u>** Sensitization, Penicillin Allergy, Fever, Serum
  Sickness, Nephritis, Anaphylactic Shock

2- Inhibition of membrane integrity

- **Polyenes:** **Colistin /Polymixen E**
- Large circular molecule consisting of a hydrophobic and hydrophilic region..
- Complex Cyclic Polypeptides
- Bactericidal,
- Used mostly against G-ve serious infe **Multiresistant**
- **Pathogens,** *Acinetobacter & Pseudor*
- Wounds, systemic.
- Topical & Intravenous,
- Nephrotoxic

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3-Inhibition Protein Synthesis

Growing polypeptide

50S portion

Chloramphenicol
Binds to 50S portion and inhibits formation of peptide bond

tRNA

Erythromycin
Binds to 50S portion, prevents translocation–movement of ribosome along mRNA

Messenger RNA

30S portion

70S prokaryotic ribosome

Translation

Streptomycin
Changes shape of 30S portion, causes code on mRNA to be read incorrectly

Tetracyclines
Interfere with attachment of tRNA to mRNA-ribosome complex

Direction of ribosome movement

- Bacterial Ribosomes composed **30s+50s=70s**

Aminoglycosides:

- Irreversibly bind to the <u>30S ribosome</u> and freeze the 30S initiation complex (30S-mRNA-tRNA)
- Bactericidal
- Broad-spectrum activity
- Mainly used against G-ve
- Not Anaerobes
- Serious Infection
- Hospital IV, IM,
- **Streptomycin, Neomycin, Amikacin, Gentamicin, Tobramycin, Netilmicin**
- **Side Effects:** Nephrotoxicity, Ototoxicity - 8th cranial nerve- hearing loss, blood-level monitoring .
- Contraindication in pregnancy causing neonatal deafness
- **Resistance:** Production acetylate, phosphorylate, adenylate enzymes
- Chromosomal & plasmid resistance
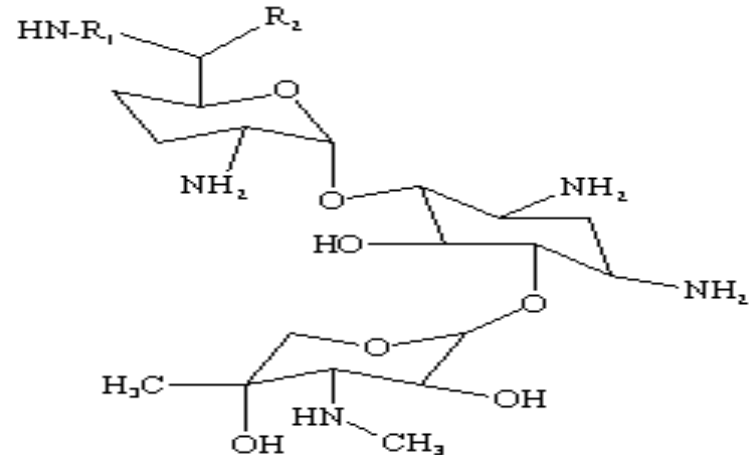
3-Inhibition Protein Synthesis

- **Tetracyclines**: <u>Mid1950s</u> :

- Bacteriostatic and broad Spectrum

- Accumulate in cytoplasmic membrane

- Inhibit essential enzymes

- Prevent attachment of the **amino-acyl tRNA to 30S ribosome complex**

- Side effect: over growth of yeast (Candida spp.)

- You NEVER give tetracyclines to pregnant women or children under 8.

- Develop of resistance by reduced active transport and Pumping efflux

- **Doxcycline, Minocycline**.. Cholera, Respiratory & Genital Infection.. *Mycoplasma, Chlamydia, Legionella* infections.. New **Tigecycline**

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- **<u>Chloramphenicol, Mid1950s</u>** :
- Bacteriostatic, inhibits protein synthesis
- Acts by binding to the **<u>50S ribosomal subunit</u>** and blocking the formation of the <u>peptide bond</u>
- Broad Spectrum
- Intracellular bacteria
- Meningitis, Septicemia, Typhoid fever,
- Highly Toxic on bone marrow

Chloramphenicol

O_2N—(benzene ring)—$\overset{H}{\underset{OH}{C}}$—$\overset{\underset{CH_2}{\overset{NH-C(=O)-CHCl_2}{|}}}{C}$—H
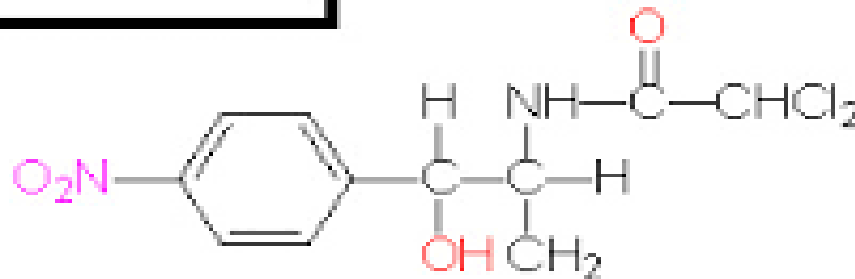
Macrolides

- **Large lactone ring structure** ranged between 14- or 16-membered rings

- Binds to the 50S ribosomal subunit

- Inhibits either peptidyltransferase activity & translocation of peptide to mRNA.

- Most widely used Macrolides .. **Erythromycin, Clarithromycin, Azithromycin** / **Orally *Long acting-12 hours)***

- Bacteriostatic, Relatively non-toxic drugs, active againstGram-positive/ Intracellular bacteria:
- Respiratory Infections.. G+ve Pneumonia, Diphtheria.., - *Streptococci- Staphylococcal , Mycoplasma, Chlamydia, Legionella pneumophila* Infections.

B) **Lincosamides/Clindamycin, Lincomycin** : inhibits protein synthesis.. Bacteriostatic .. _Staphylococcus_.. Streptococci.. Bones, Oral cavity.. Anaerobic Infections.

* Common cause **Pseudomembranous colitis**.. Serious bloody diarrhea.. Due to increase growth anaerobic spore-forming _Clostridium difficile_ in intestine.

- **Fusidic acid**:
- Bacteriostatic , Fusidic acid inhibits protein synthesis, used against staphylococcal skin infection.

Inhibition Nucleic Acid Synthesis-4

- **Nalidixic acid (Quinolone):** Inhibit **DNA Gyrase/** Replication.. Bactericidial.

- **Floroquinolones:** (1980s-2000s).. inhibit **DNA Gyrase** & **transcription**. **Bactericidal**, Norfloxacin, Ciprofloxacin, Levofloxacin , Ofloxacin..Broad spectrum.. More G-ve than G+e Infections.. intracellular pathogens, Urinary Tract, Pneumonia, Septicemia.. Resistance by altered DNA gyrase.. Develop due to mutation during treatment.

- **Nitrofurantoin** : Damage DNA.. Bacteriostatic

- Both synthetic drugs are active against G-ve enteric bacteria..*E.coli..* used in Urinary tract Infection.

- **Rifamycin /Rifampin**: binds to the **RNA polymerase..** Prevent its transcription from DNA .. **Bactericidal**, *Mycobacteria*.. Intracellular bacteria.. *Chlamydia, Brucella*, Resistance due to change in RNA polymerase ß-subunit .

5-Inhibition Synthesis of Essential Metabolites

- **Sulfa drugs / Sulfonamides** : Structure analogue to PABA.. Compete with it .. **Block folic acid synthesis**.. Essential for nucleic acid synthesis Mammals don't need PABA or its analogs
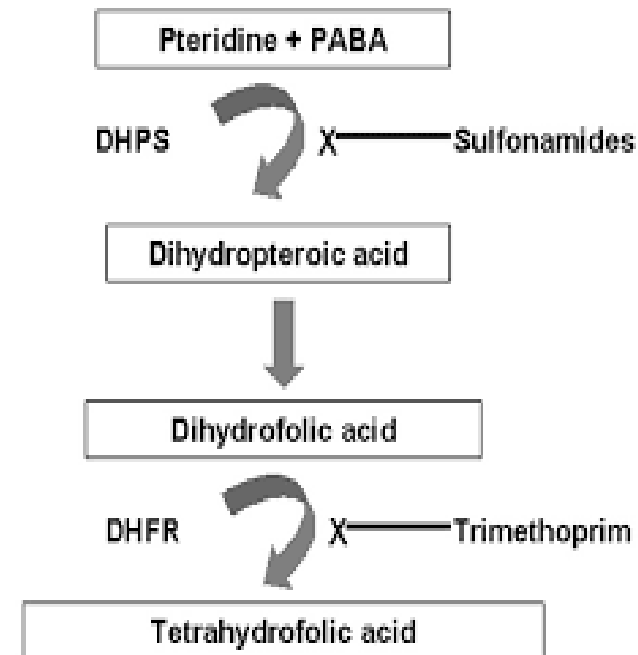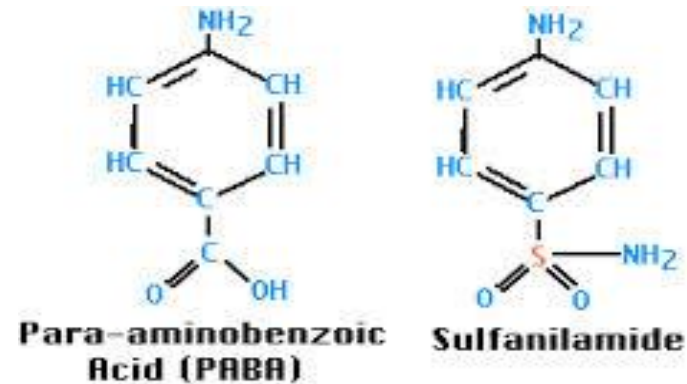
- **Bacteriostatic**.. Now Rare used alone, Rapid develop Resistance by altered enzyme that is no longer inhibitable by sulfonamides.

- **Sulfamethoxazole-trimethoprim**
- (Cotrimoxazole).. Combined effects/Synergism..
- Broad Spectrum,
- UTI, RTI

Para–aminobenzoic Acid (PABA)   Sulfanilamide

Pteridine + PABA

DHPS → X ——— Sulfonamides

Dihydropteroic acid

Dihydrofolic acid

DHFR → X ——— Trimethoprim

Tetrahydrofolic acid

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- **Antituberculosis Drugs:**
- Inhibition <u>Mycolic acid</u> ..Part of Mycobacterial Cell Wall.. *Mycobacterium tuberculosis*.
- **Isoniazid** (INH), **Ethambutol**, **Cycloserine**, **Rifampin, Streptomycin**, 6- months treatment..always combination 2-3 drugs.
- Rapid Resistance if used alone .
- Treatment of R-tuberculosis 1-2 years.

- **Metronidazol**: Active against most Anti-protozoa & Most Anaerobic Bacteria.

Antibiotic Susceptibility Tests

- Laboratory Antibiotic Susceptibility Tests:

- Culture, Isolation, Identification of Bacteria from clinical specimen as pure E. coli, S. aureus,

- Testing of only one pure fresh bacteria culture on Mueller-Hinton Broth & Agar.. Disk Diffusion test .. Measure inhibition zone after 24 hrs incubation 37°C

- Minimal Inhibitory Concentration (MIC/ug/ml) .. E-test

  consists of a strip containing an exponential gradient of one antibiotic(1-2-4-8-16-32-64-128-256) ug/ml

- Lab Report: Susceptible isolates (S) .. Intermediate susceptible (IS).. Resistant (R)

- Multi-resistant.. Resistance to $\geq$2 antibiotic classes.

Antibiotic Disc -Test
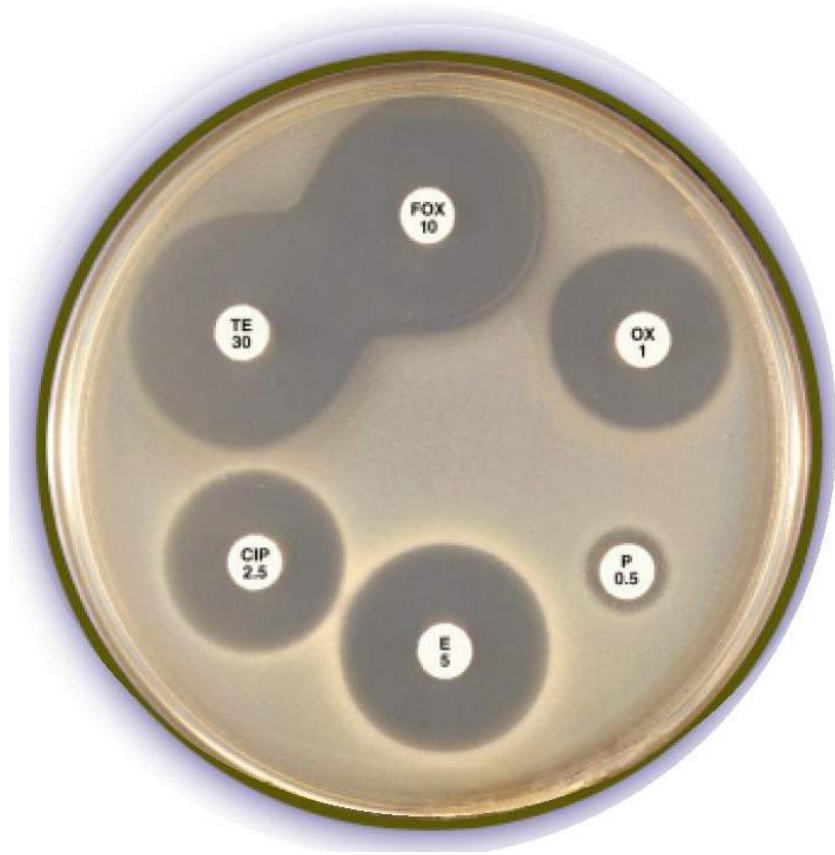
Antibiotic E-test (MIC-mg/ml)

Antimicrobial Resistance

- **Resistance** is becoming a serious problem Worldwide.. more commensal /pathogenic microorganisms (Bacteria, Yeast, Viruses) are become untreatable with commonly used antimicrobials.. *Acinetobacter spp*., *Pseudomonas spp*., *MR-staphylococci* (MRSA), Va-R Enterococcus, MR-*Mycobacteria spp*… **High Mortality & High Treatment Cost** .

- This problem is due to over use/ misuse of antimicrobials in medicine & agriculture and misuse by general population.

- **Antibacterial resistance** including β-lactamases, efflux pumps, porin mutations, modifying enzymes and binding site mutations. Horizontal transfer of combined resistance by plasmids. Develop multidrug resistance.. **Mostly Not Reversible**.

- Antibiotics selective Pressure. Human, Animals, Environment.
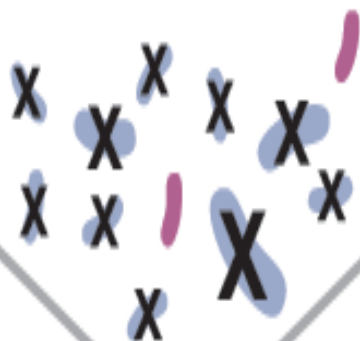
How Antibiotic Resistance Happens

1.
Lots of germs.
A few are drug resistant.

2.
Antibiotics kill bacteria causing the illness, as well as good bacteria protecting the body from infection.
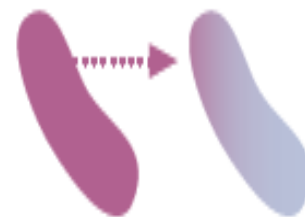
3.
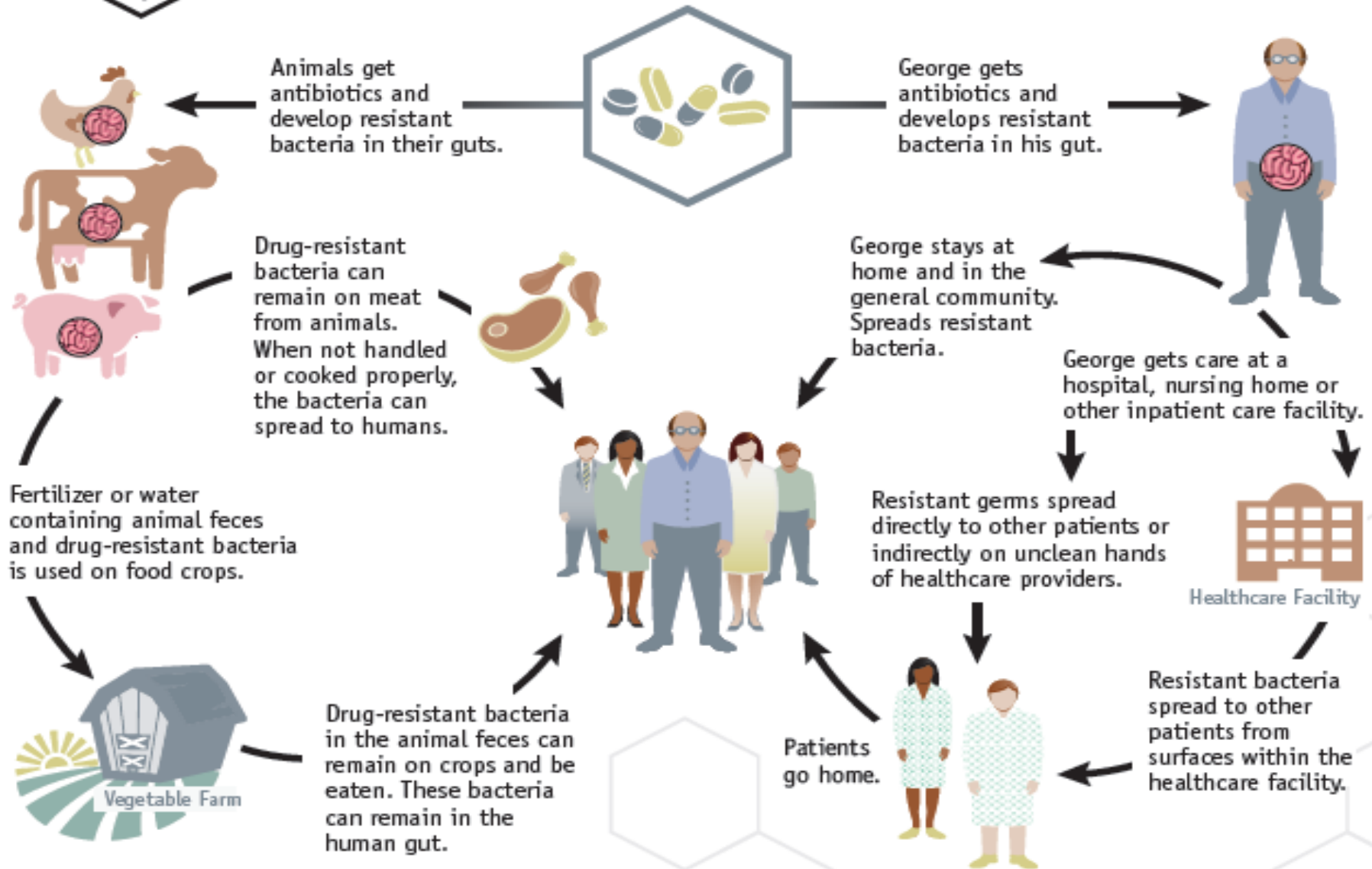The drug-resistant bacteria are now allowed to grow and take over.

4.
Some bacteria give their drug-resistance to other bacteria, causing more problems.

Examples of How Antibiotic Resistance Spreads

Animals get antibiotics and develop resistant bacteria in their guts.

George gets antibiotics and develops resistant bacteria in his gut.

Drug-resistant bacteria can remain on meat from animals. When not handled or cooked properly, the bacteria can spread to humans.

George stays at home and in the general community. Spreads resistant bacteria.

George gets care at a hospital, nursing home or other inpatient care facility.

Fertilizer or water containing animal feces and drug-resistant bacteria is used on food crops.

Resistant germs spread directly to other patients or indirectly on unclean hands of healthcare providers.

Healthcare Facility

Vegetable Farm

Drug-resistant bacteria in the animal feces can remain on crops and be eaten. These bacteria can remain in the human gut.

Patients go home.

Resistant bacteria spread to other patients from surfaces within the healthcare facility.

Simply using antibiotics creates resistance. These drugs should only be used to treat infections.