# ANTIMICROBIALS

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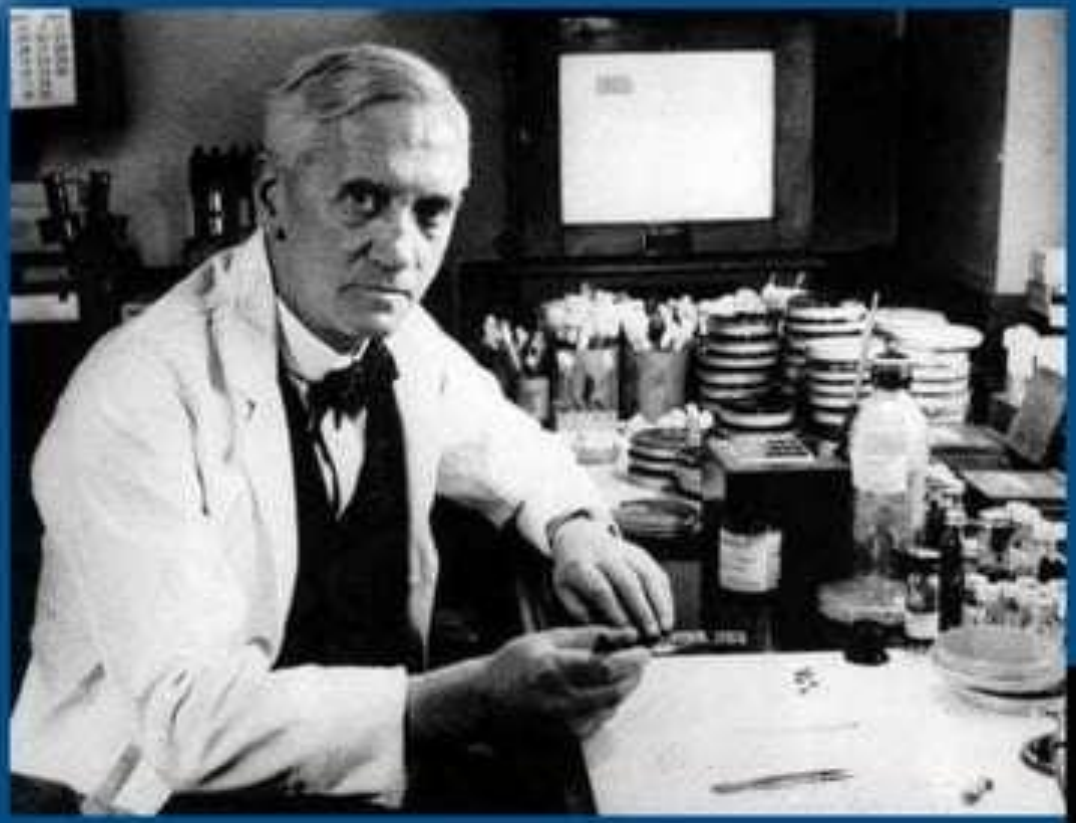
Asst Prof Microbiology

# Fleming and Penicillin



*P. chrysogenum* destroyed *S. aureus* 1928

Rapidly Increasing Antibiotic Resistance Constitutes One of the Most Important Clinical, Epidemiological and Microbiological Problems of Today

# What is an Antimicrobial?

A substance of biological, semi-synthetic or synthetic origin, produced by a fungus or bacterium as secondary metabolites, that inhibits or stops growth of other microorganisms *in vitro* and *in vivo* selectively, when used in low concentration

- **Antibiotic**

  Chemical produced by a microorganism that kills or inhibits the growth of another microorganism

- **Antimicrobial agent**

  Chemical that kills or inhibits the growth of microorganisms

- **Selective toxicity**

  A drug that kills harmful microbes without damaging the host

- **Therapeutic index**

  Toxic dose/ Effective dose

# Microbial Sources of Antibiotics

| TABLE 20.1 | Representative Sources of Antibiotics |
|---|---|

| Microorganism | Antibiotic |
|---|---|
| **Gram-Positive Rods** | |
| *Bacillus subtilis* | Bacitracin |
| *Bacillus polymyxa* | Polymyxin |
| **Actinomycetes** | |
| *Streptomyces nodosus* | Amphotericin B |
| *Streptomyces venezuelae* | Chloramphenicol |
| *Streptomyces aureofaciens* | Chlortetracycline and tetracycline |
| *Streptomyces erythraeus* | Erythromycin |
| *Streptomyces fradiae* | Neomycin |
| *Streptomyces griseus* | Streptomycin |
| *Micromonospora purpureae* | Gentamicin |
| **Fungi** | |
| *Cephalosporium* spp. | Cephalothin |
| *Penicillium griseofulvum* | Griseofulvin |
| *Penicillium notatum* | Penicillin |

# The Ideal Drug

- Selective toxicity against target pathogen but not against host
- Bactericidal vs. bacteriostatic
- Favorable pharmacokinetics
  - Reach target site in body with effective concentration
- Spectrum of activity
  - Broad vs. narrow
- Lack of "side effects"
- Therapeutic index
  - Effective to toxic dose ratio
- Little resistance development
- Adverse effect profile
- Cost

*** There is no perfect drug**

# Use of Antimicrobial Therapy

- **Prophylaxis**

  Prevention of infection to which patient is felt to be at high risk of acquiring

- **Empiric**

  Target empiric therapy to likely pathogens and local antibiogram

- **Definitive**

  Target definitive therapy to known pathogens and antimicrobial susceptibility results

# Terminology (1)

- **Antibacterial spectrum**

  Range of activity of an antibiotic against microorganisms

- **A broad spectrum**

  Antibiotic that can inhibit wide range of Gram positive and Gram negative bacteria

  (Carbapenems, 3-4th generation cephalosporins, quinolones)

- **A narrow spectrum**

  Antibiotic that is active only against a limited number of bacteria

  (Penicillin G)

# Terminology (2)

- **Bacteriostatic activity**

  Level of antimicrobial activity that inhibits the growth of bacteria

- **Bactericidal activity**

  Level of antimicrobial activity that kills the bacteria

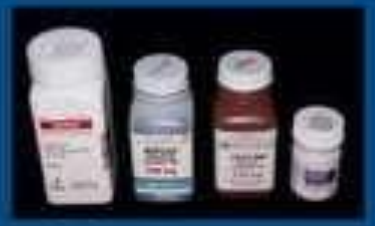
- **Minimum inhibitory concentration (MIC)**

  The lowest concentration that inhibits the growth of bacterial population

- **Minimum bactericidal concentration (MBC)**

  The lowest concentration that kills 99.9% of the bacterial population

# Terminology (3)



- **Antibiotic combinations**
- **Antibiotic synergism**

  Combination of antibiotics have enhanced activity when tested together compared with each antibiotic alone (e.g. 2 + 2 = 6)

  (Ampicillin+gentamicin in entercoccal carditis)
- **Additive effect**

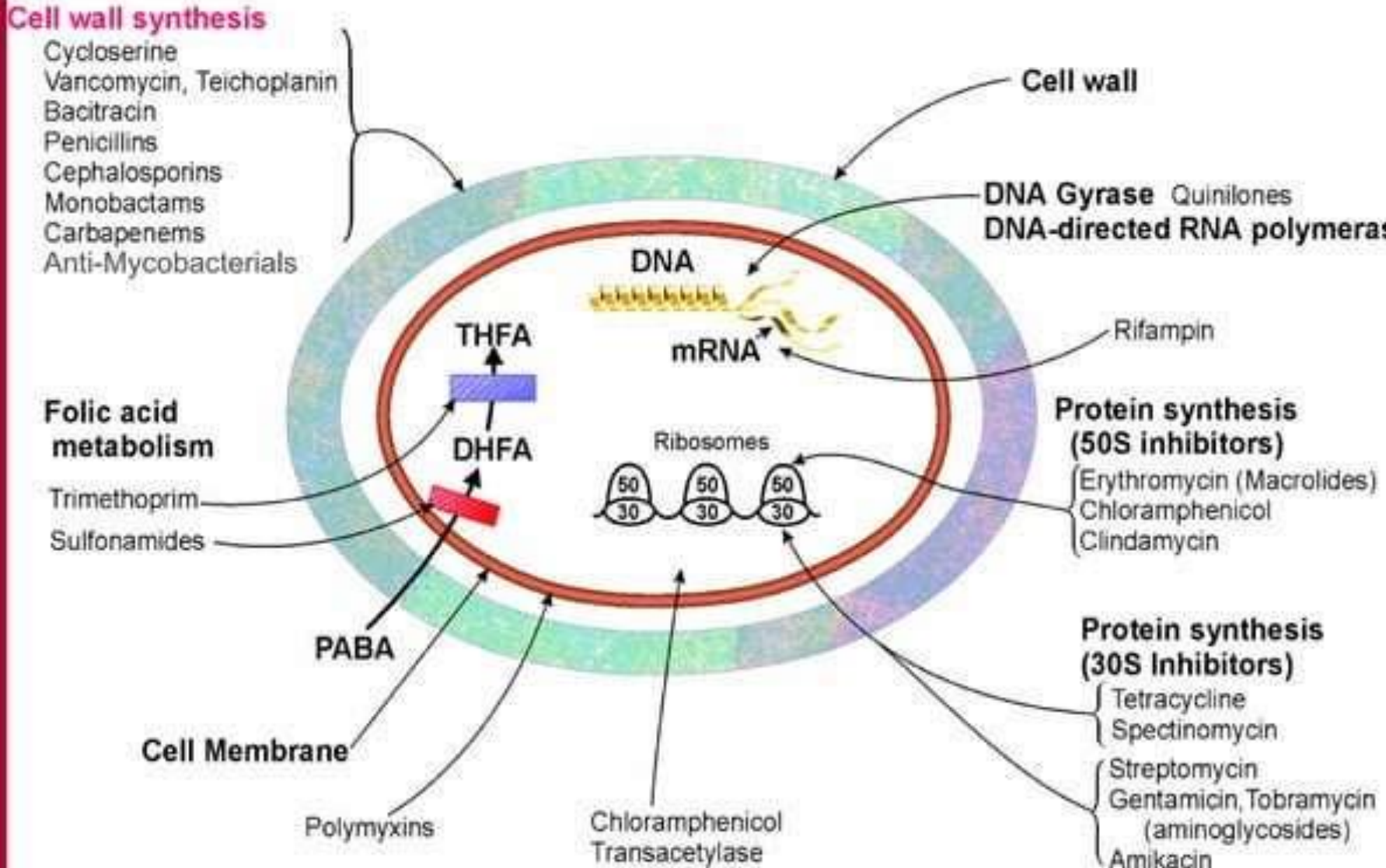
  Combination of antibiotics has an additive effect (e.g. 2 + 2 = 4)

  (Combination of two β-lactam antibiotics)
- **Antibiotic antagonism**

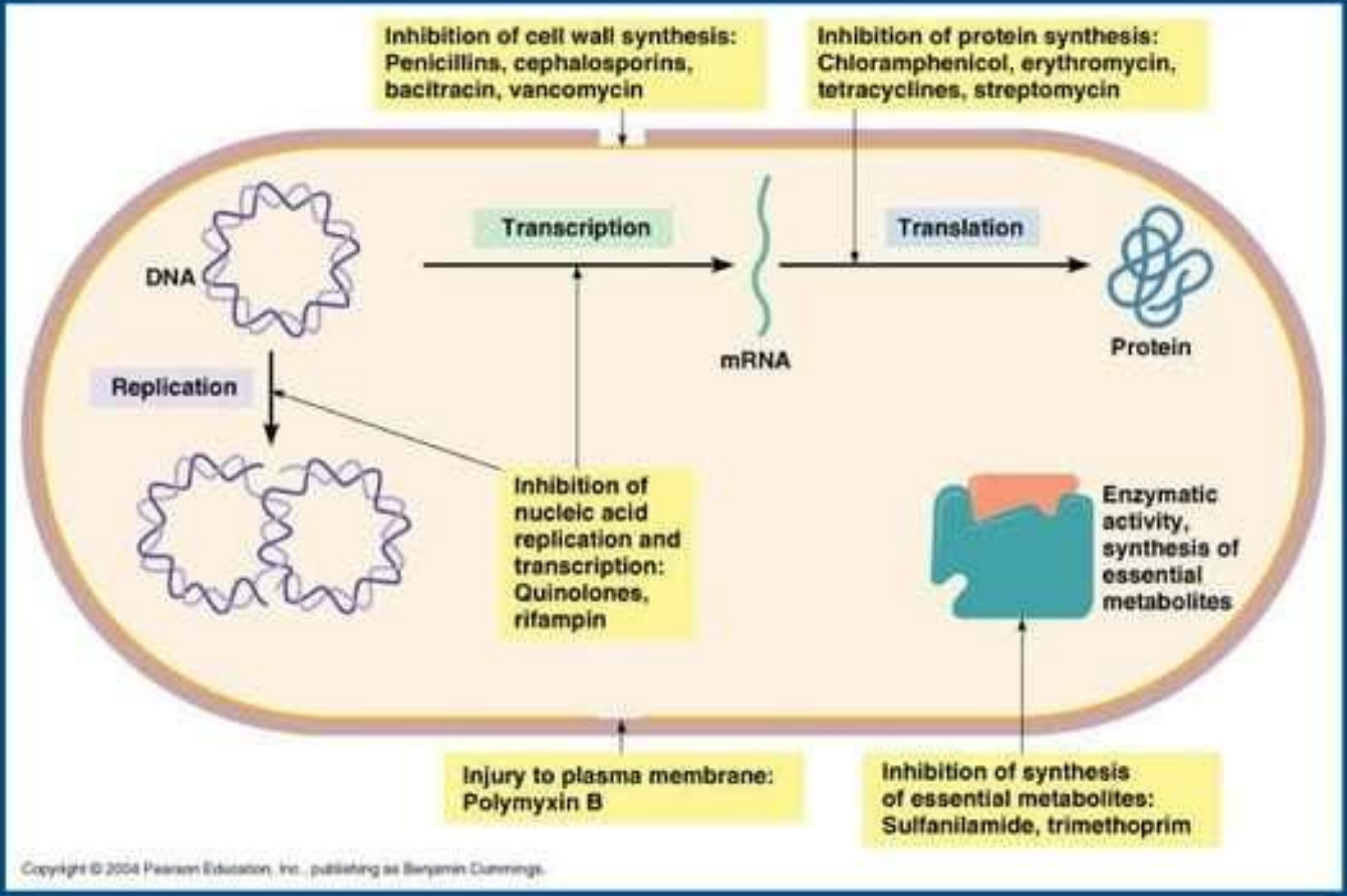
  Combination in which the activity of one antibiotic interferes with the activity of the other (e.g. 2 + 2 < 4)

# Antibiotics - Mechanisms of Action

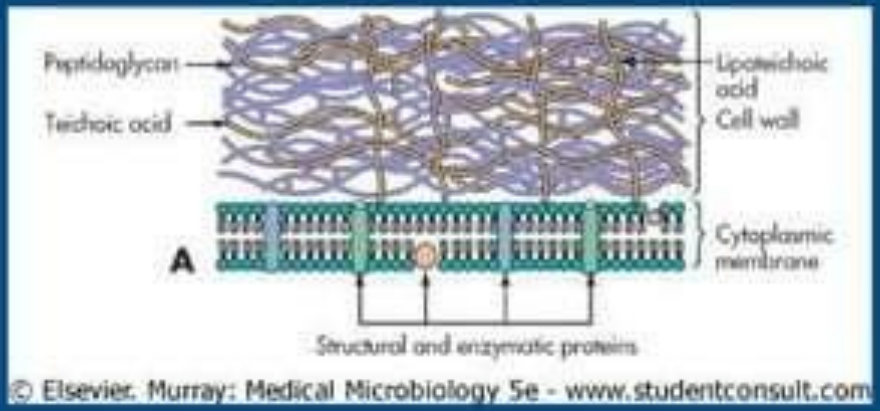
## Bacterial Targets for Current Antibiotics Used in the Clinic

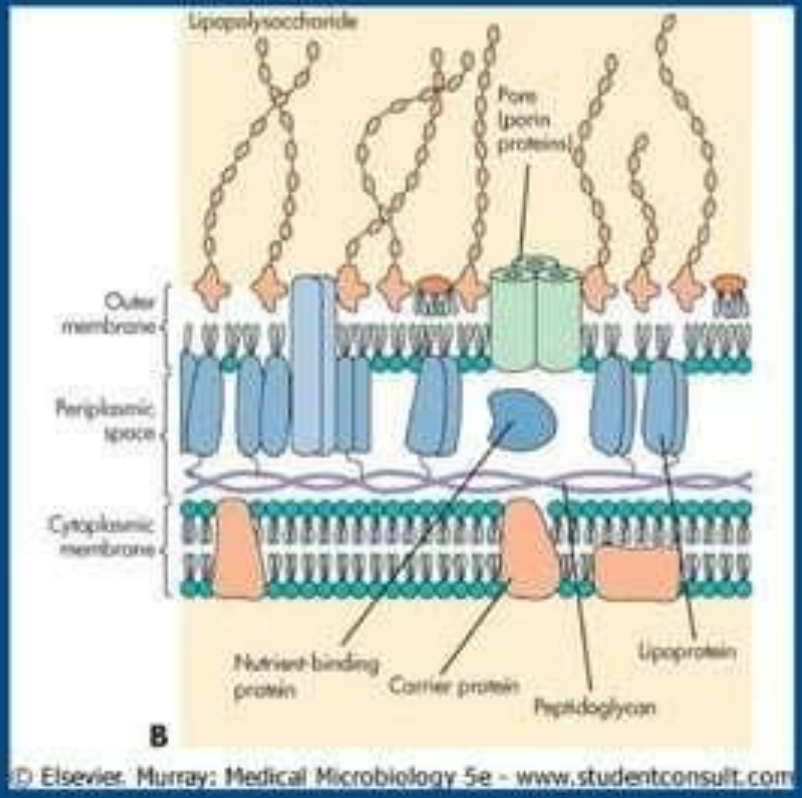


# Antibiotics - Mechanisms of Action

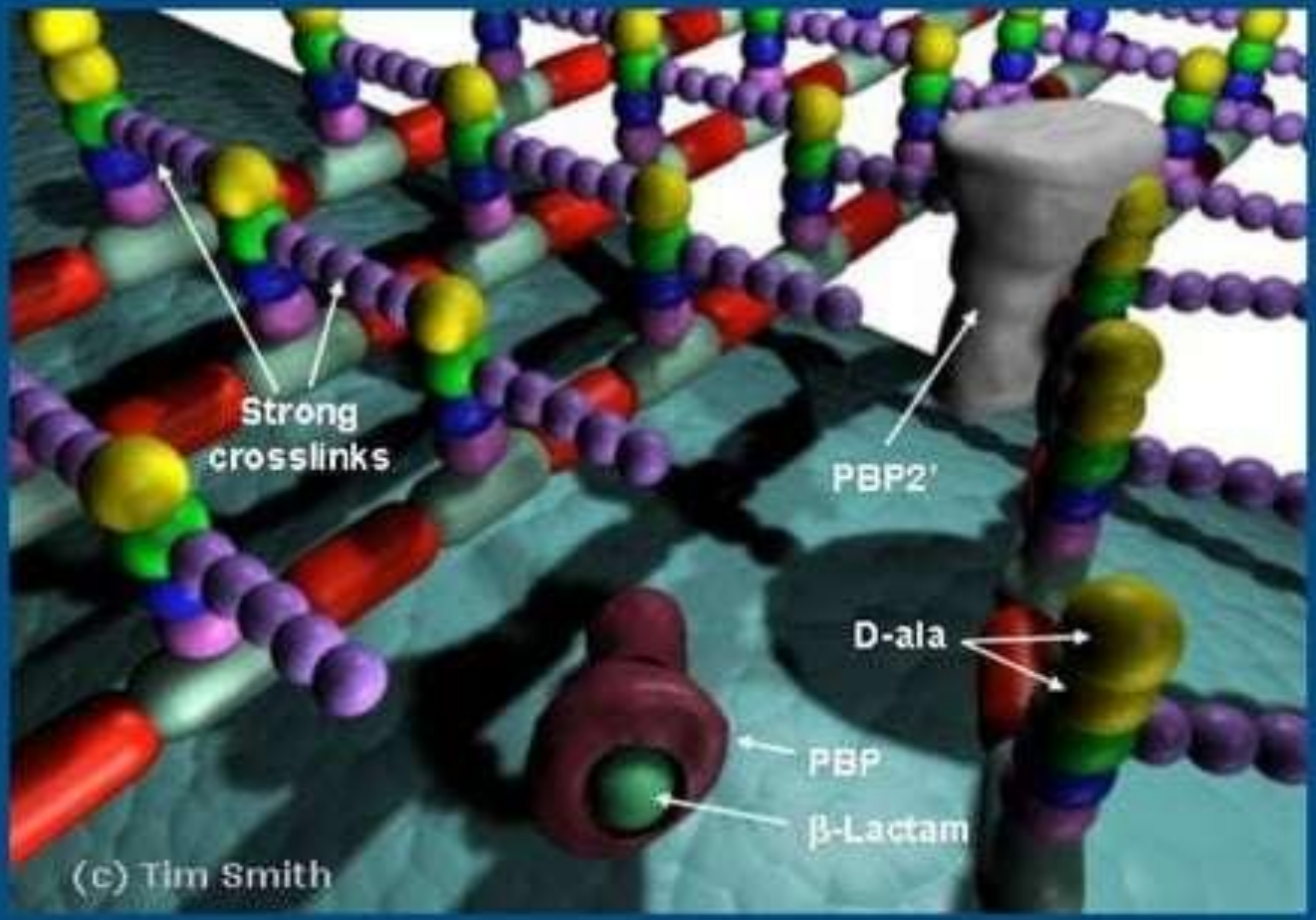


# Gram-positive and Gram-negative cell wall



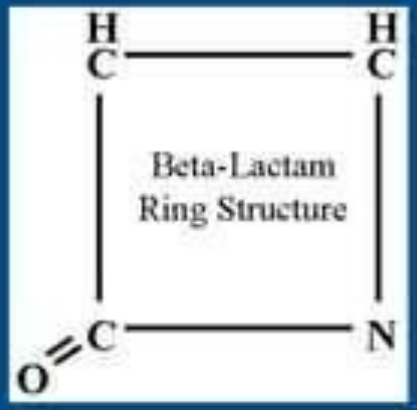
# Structure of Peptidoglycan

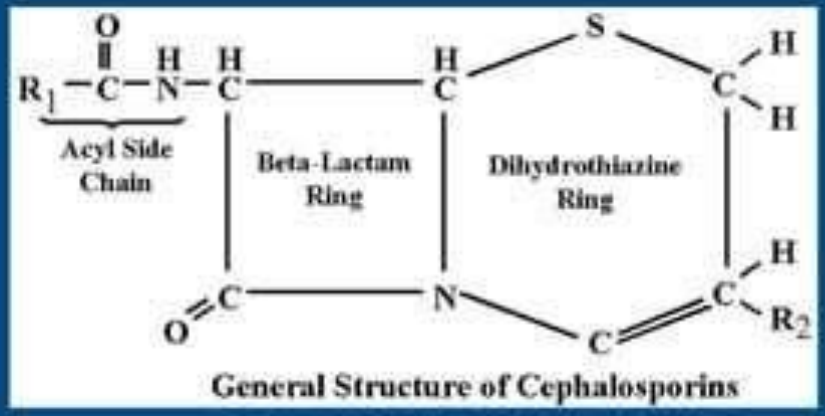



β-lactams inhibit synthesis of crosslinks

# Cell Wall Synthesis Inhibitors

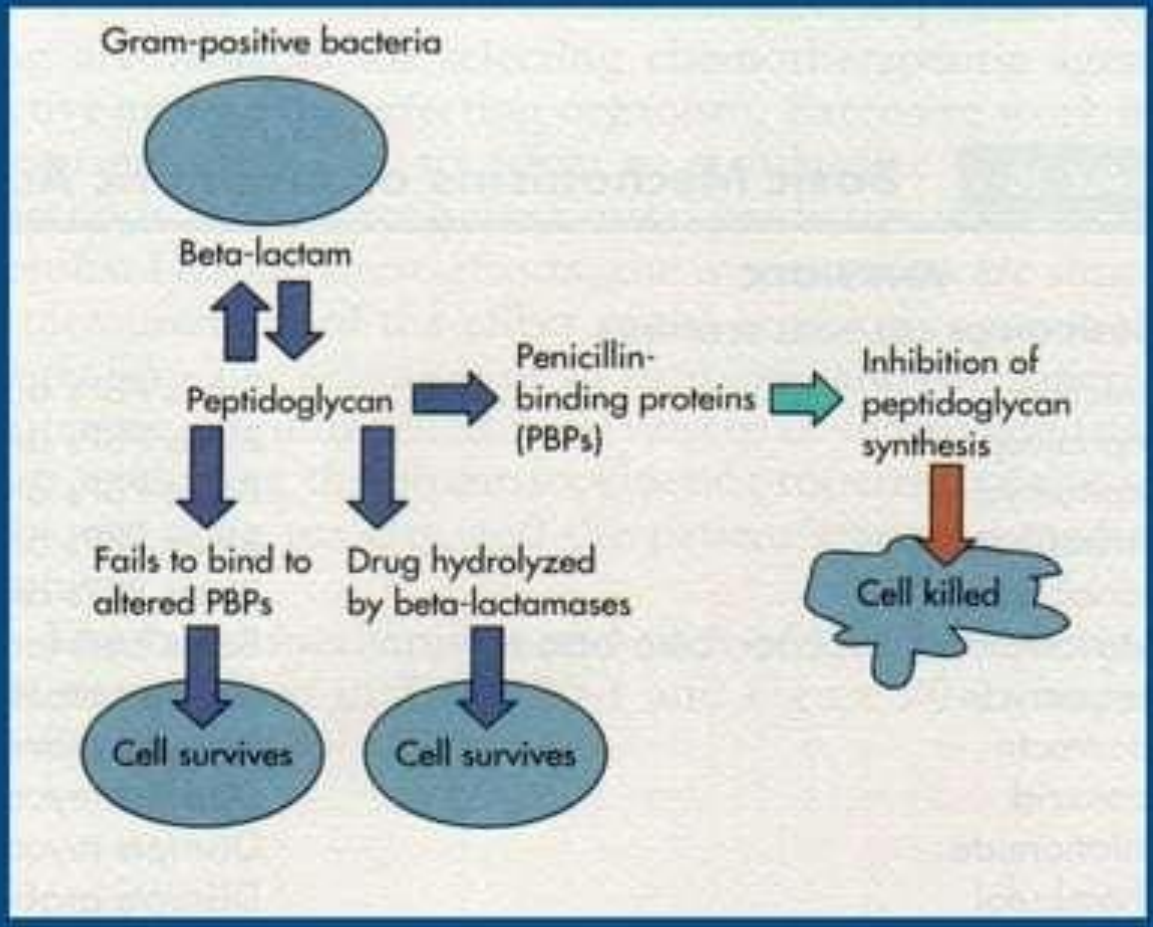
β-Lactam ring structure



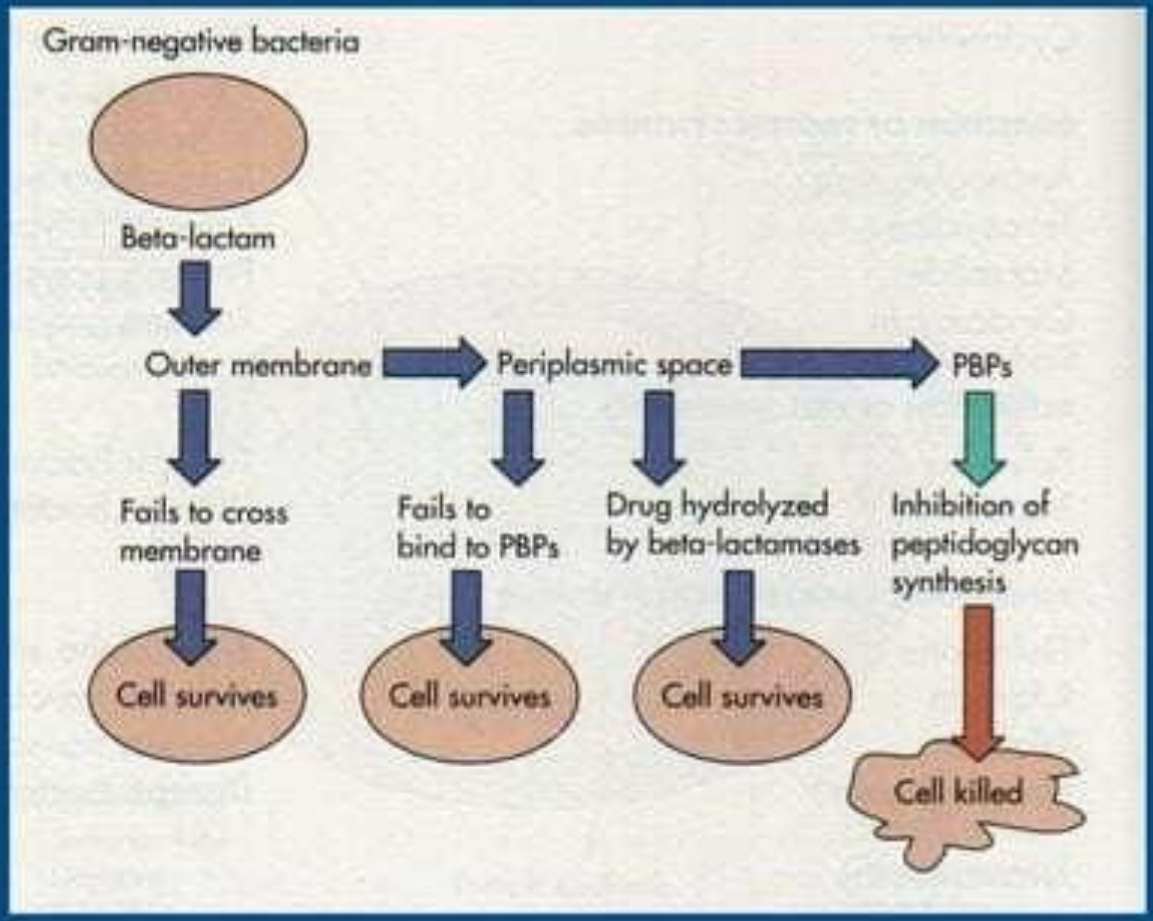




General Structure of Cephalosporins

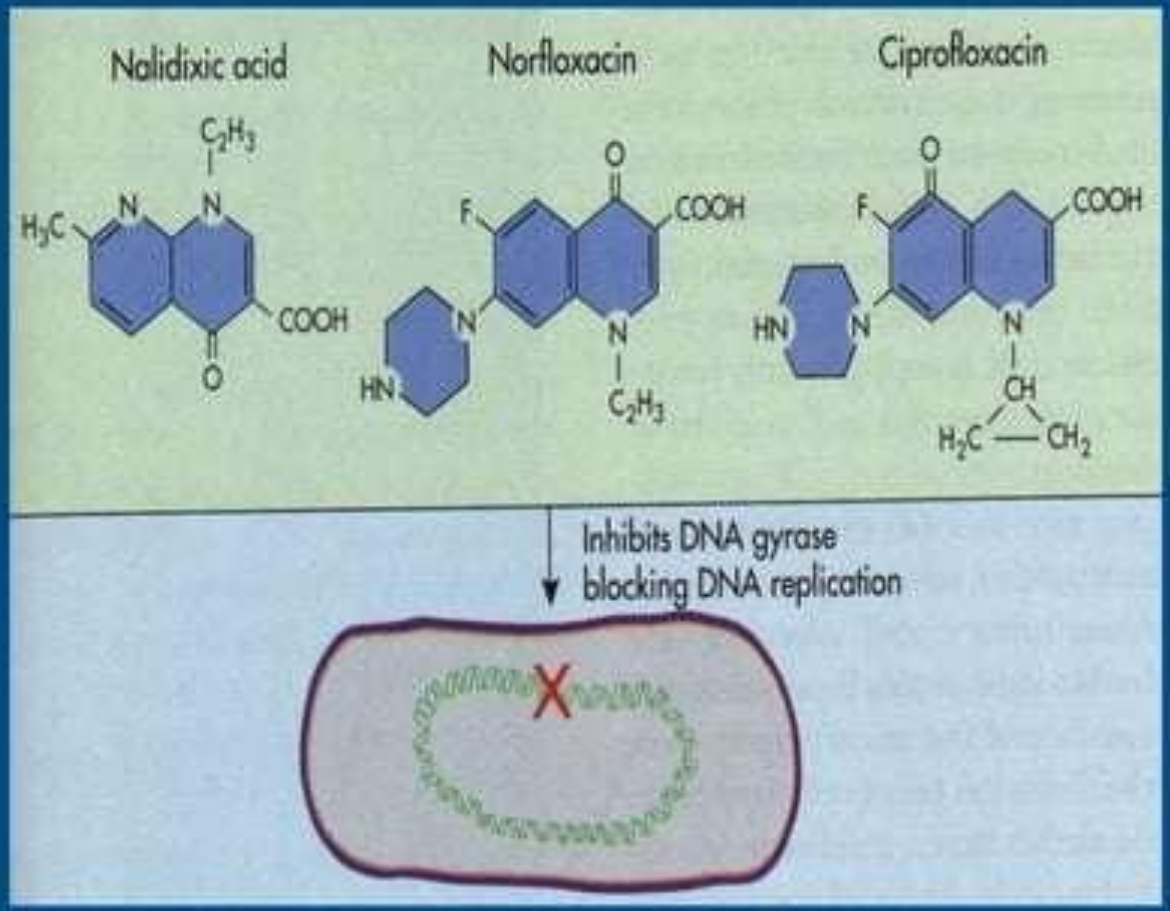
# Exposure to β-Lactams – Gram pos.



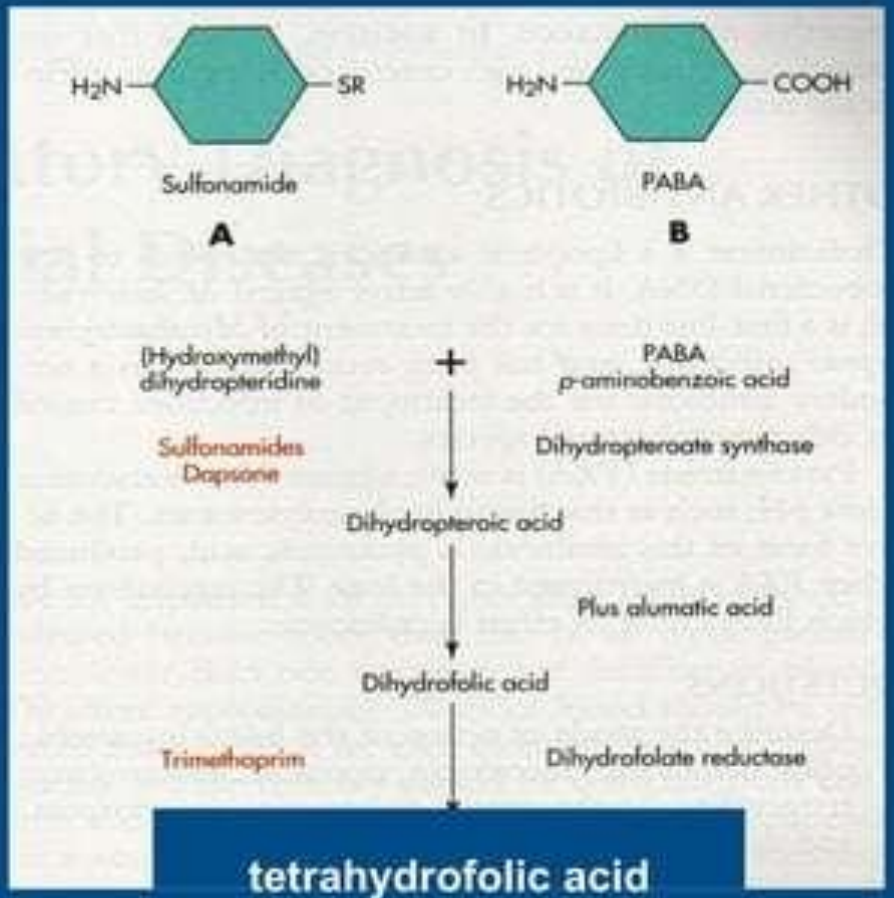
# Exposure to β-Lactams – Gram neg.



# Inhibition Of DNA Synthesis



# Anti-metabolite Action



# Clinical Uses

| PATHOGENS | TYPICAL DRUG |
|---|---|
| **Gram positive** | |
| Pen-ase (-) | Penicillin G (oral or IM) |
| Pen-ase (+) | Methicillin, Nafcillin |
| **Gram negative** | |
| Enterics, etc. | Ampicillin, gentamicin, etc. |
| *Pseudomonas* | Ticarcillin, tobramycin |
| *B. fragilis* | Clindamycin |

| PATHOGENS | TYPICAL DRUG |
| --- | --- |
| **Mycobacterium** | Streptomycin, rifampicin<br>Iso-nicotinic hydrazide (INH) |
| **Fungi**<br>Cutaneous<br>Deep | <br>Nystatin<br>Amphotericin B, ketoconazol |
| **Parasites**<br>Plasmodium<br>Giardia | <br>Chloroquine<br>Metronidazole, Quinacrine |

# ANTIBIOTIC RESISTANCE

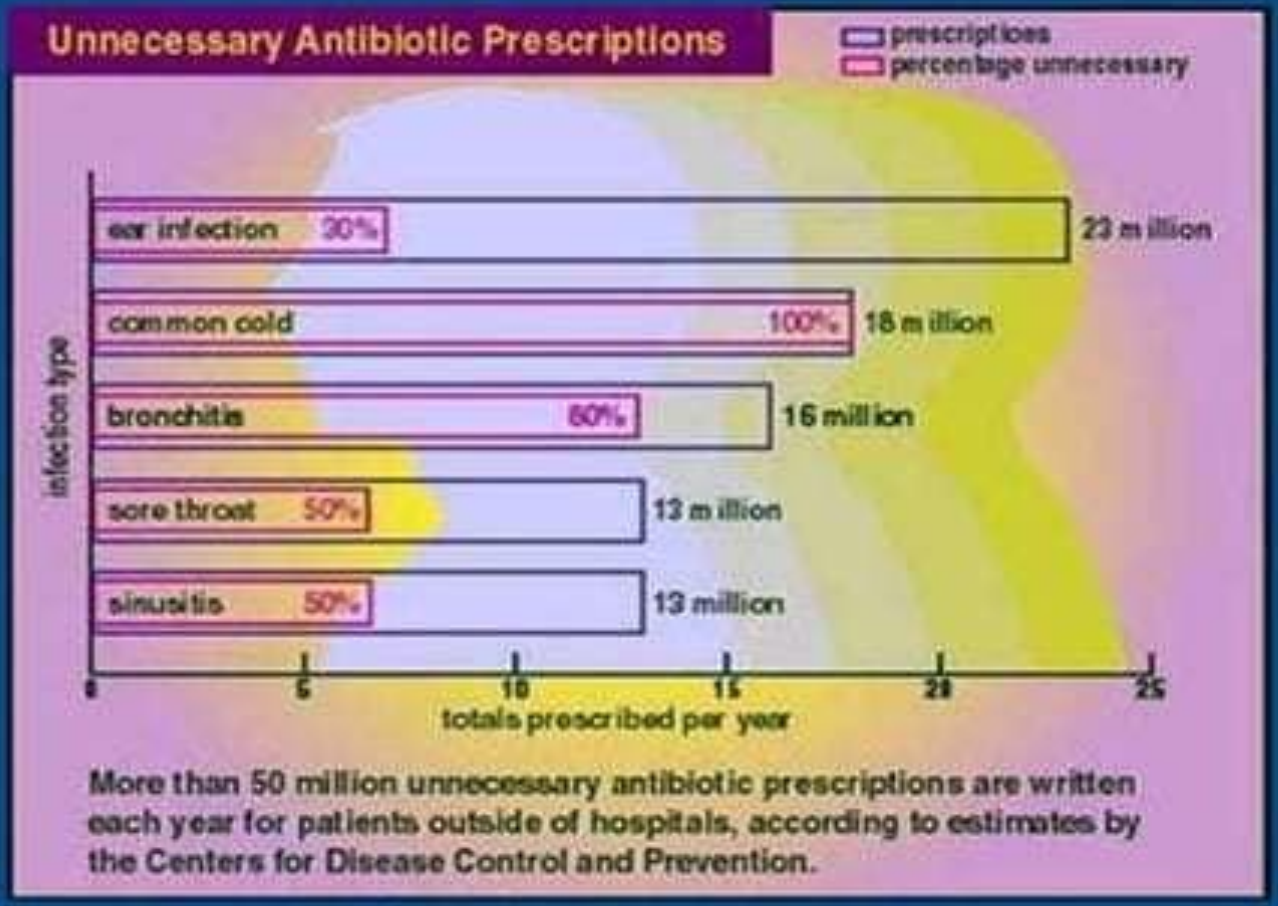
# The Problem of Antibiotic Resistance

- Penicillin resistance first identified in 1940's
- Since then, antibiotic resistance has developed faster than new drugs
- Estimated cost of infections: $4-5 million per year
- Antibiotic resistance previously concentrated in hospitals, especially ICUs
- MRSA recently estimated to kill 18,000 Americans yearly

# Examples of overuse/misuse

- Empiric use (no known etiologic agent)
- Increased use of broad spectrum agents
- Prescription not taken correctly
- Antibiotics sold without medical supervision
- Prophylactic use before surgery
- Antibiotics for viral infections
- Spread of resistant microbes in hospitals due to lack of hygiene
- Patients who do not complete course (TB, AIDS)
- Antibiotics in animal feeds

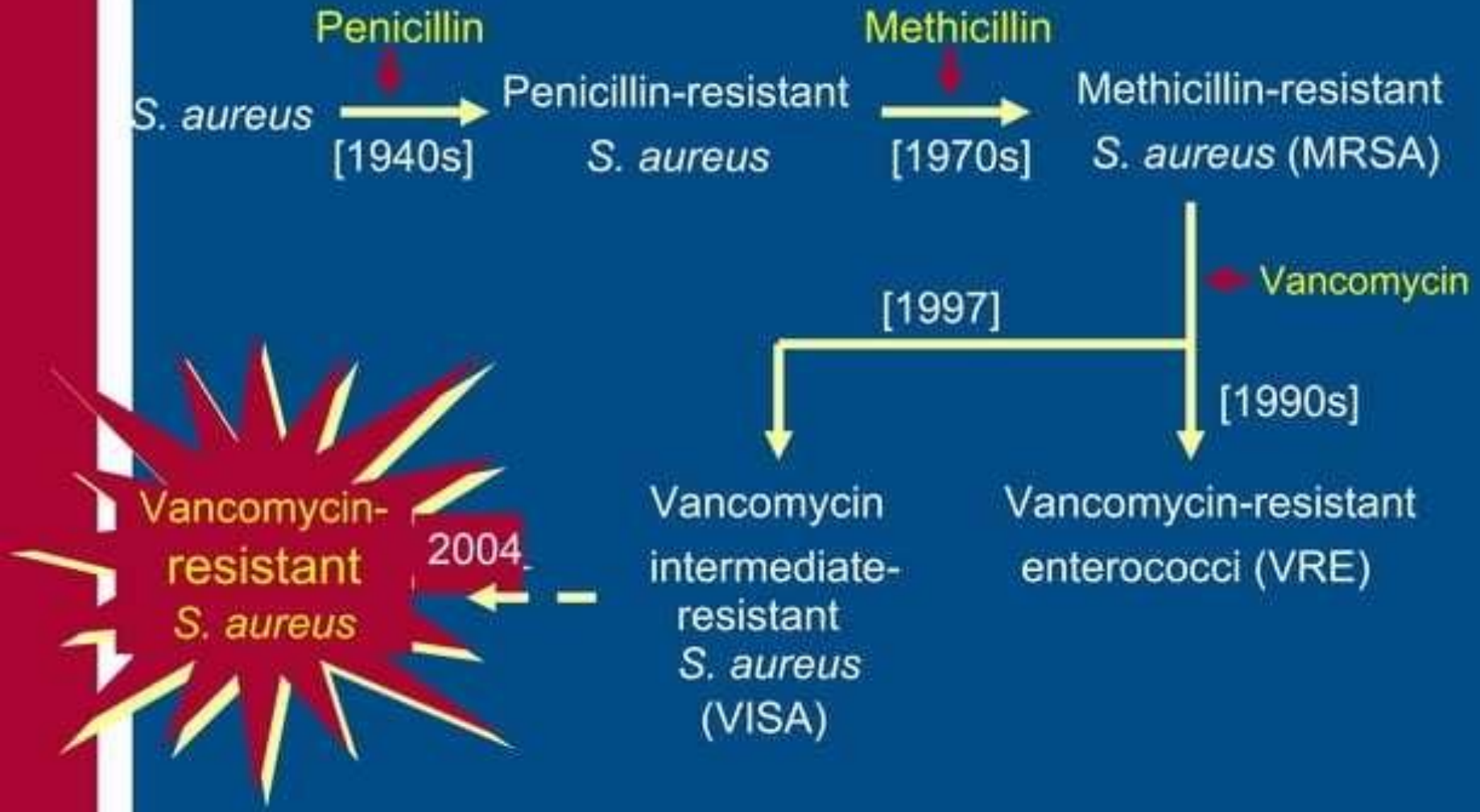
# Unnecessary Antibiotic Prescriptions



# Antibiotic Resistant Microorganisms

- **MRSA:** methicillin-resistant *S. aureus*
- **VRE:** vancomycin-resistant enterococci
- **VRSA & VRSE:** vancomycin-resistant *S. aureus* and *S. epidermidis*
- **VISA:** vancomycin intermediately susceptible *S. aureus*
- **GISA:** glycopeptide intermediately susceptible *S. aureus*
- **PRSP:** penicillin-resistant *S. pneumoniae*
- **QRNG:** quinolone-resistant *N. gonorrhoeae*
- **ESBL producer:** extended spectrum β-lactamase producer

# Evolution of Drug Resistance in *S. aureus*



# Terminology (4)

- **Resistant organism**

  MICs of organism are higher than achieved drug concentrations in tissues

- **Intermediately resistant**

  The antibiotic may still be effective but higher doses should be used

- **Highly resistant**

  The antibiotic tissue concentrations are likely not to exceed MICs of the microorganisms

# Terminology (5)

- **Bacteriophage**

  Virus, infecting bacteria (virus of bacteria)
- **Integron**

  Slice(s) of DNA, cassette of gene that may be entered into other cell
- **Plasmid (R factors)**

  Circular double stranded DNA molecule, located separately of the chromosomal RNA
- **Transposon**

  Genes moving from one point to another (jumping genes)

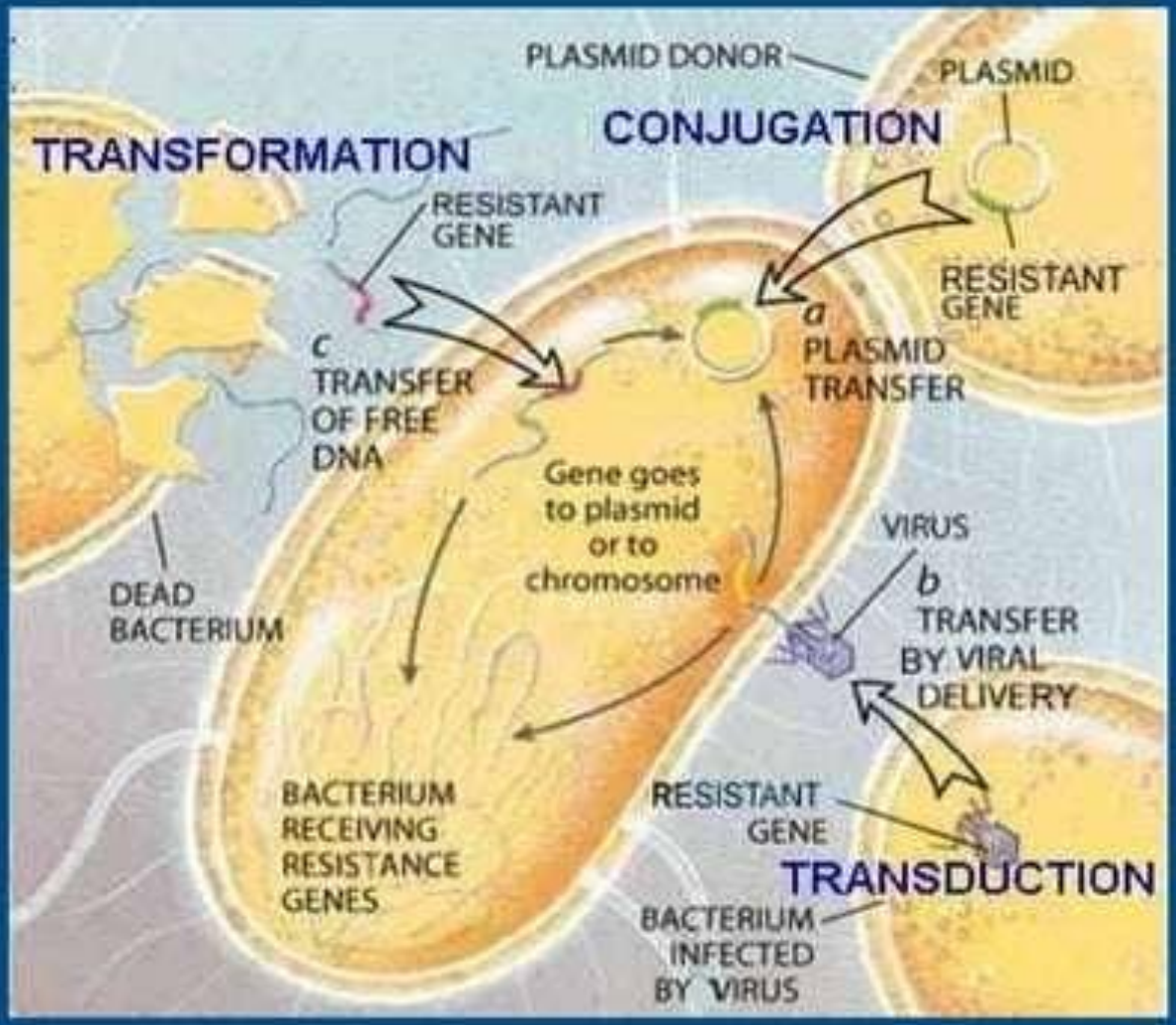
# Antibiotic Resistance

**Types of resistance**

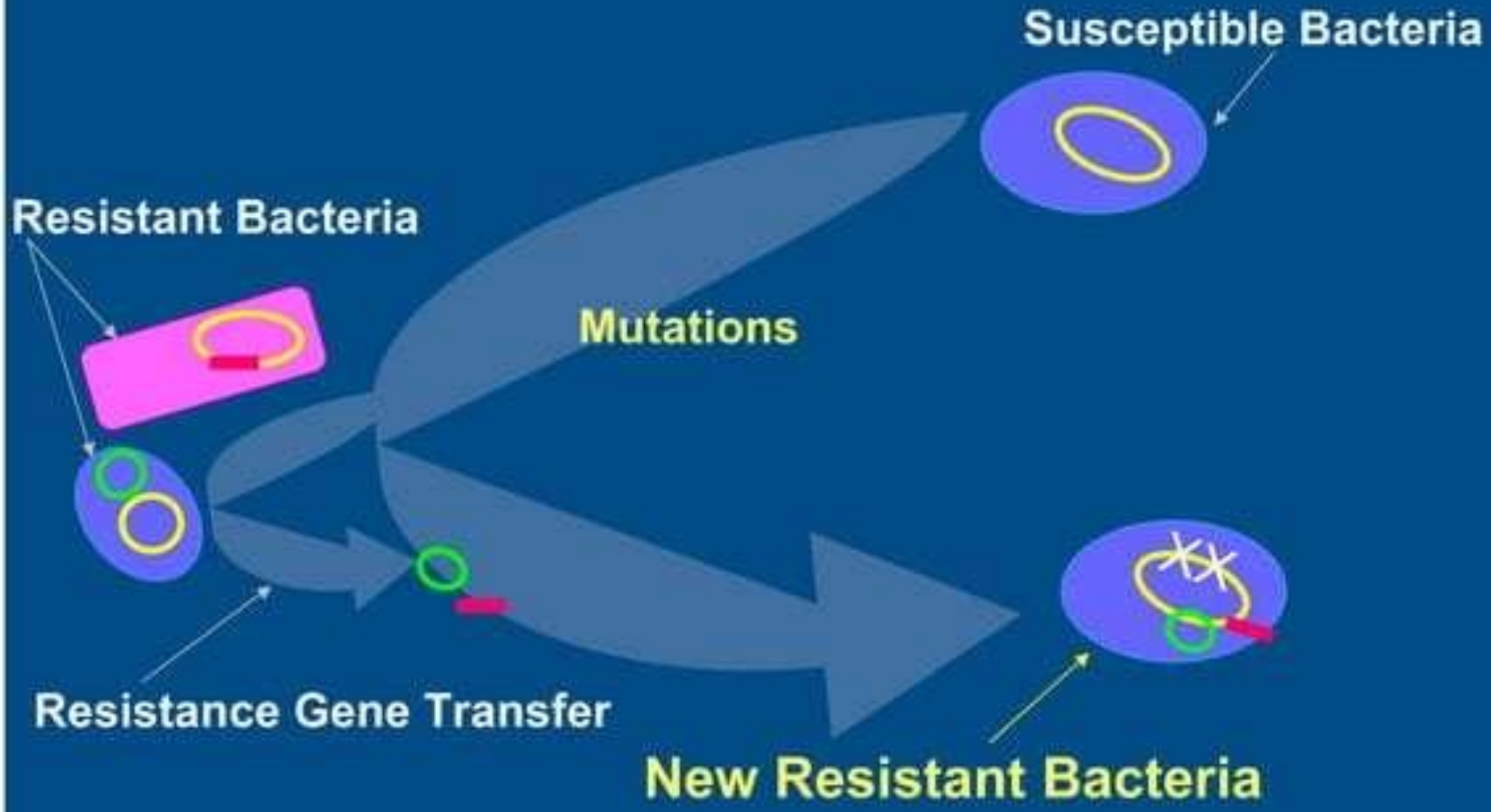
- **Intrinsic** or natural resistance
  - G-neg bacteria are resistant to vancomycin (large molecule)
  - Lack of transport system (Xanthomonas)
  - Lack of target (Mycoplasma)
- **Acquired resistance**
  - Mutations (PBP) - spontaneous
  - Acquisition of foreign DNA
    - Plasmid exchange
    - Transposons

- Conjugation
- Transduction
- Transformation

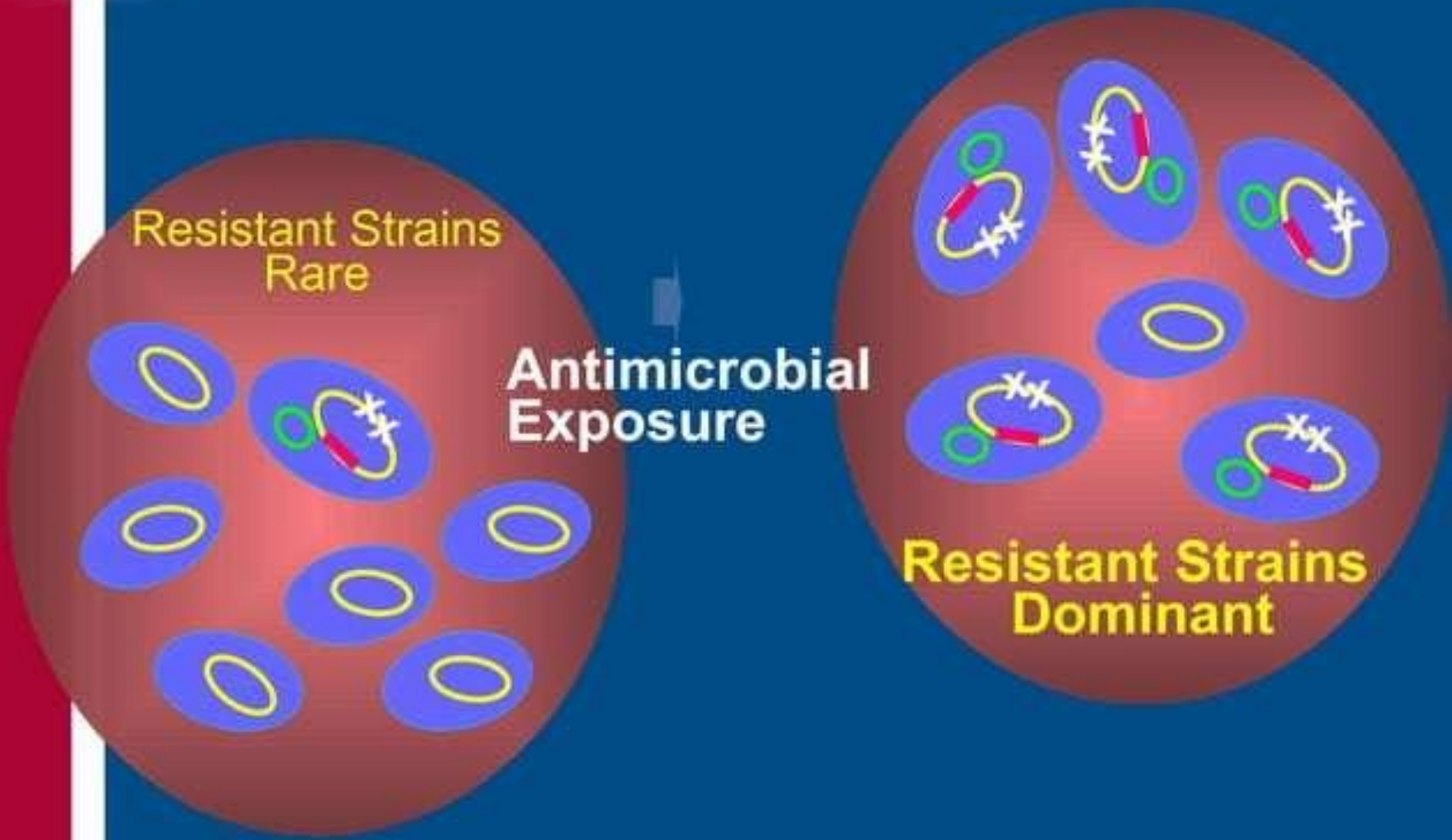
# Mechanisms of Resistance Gene Transfer



# Emergence of Antimicrobial Resistance

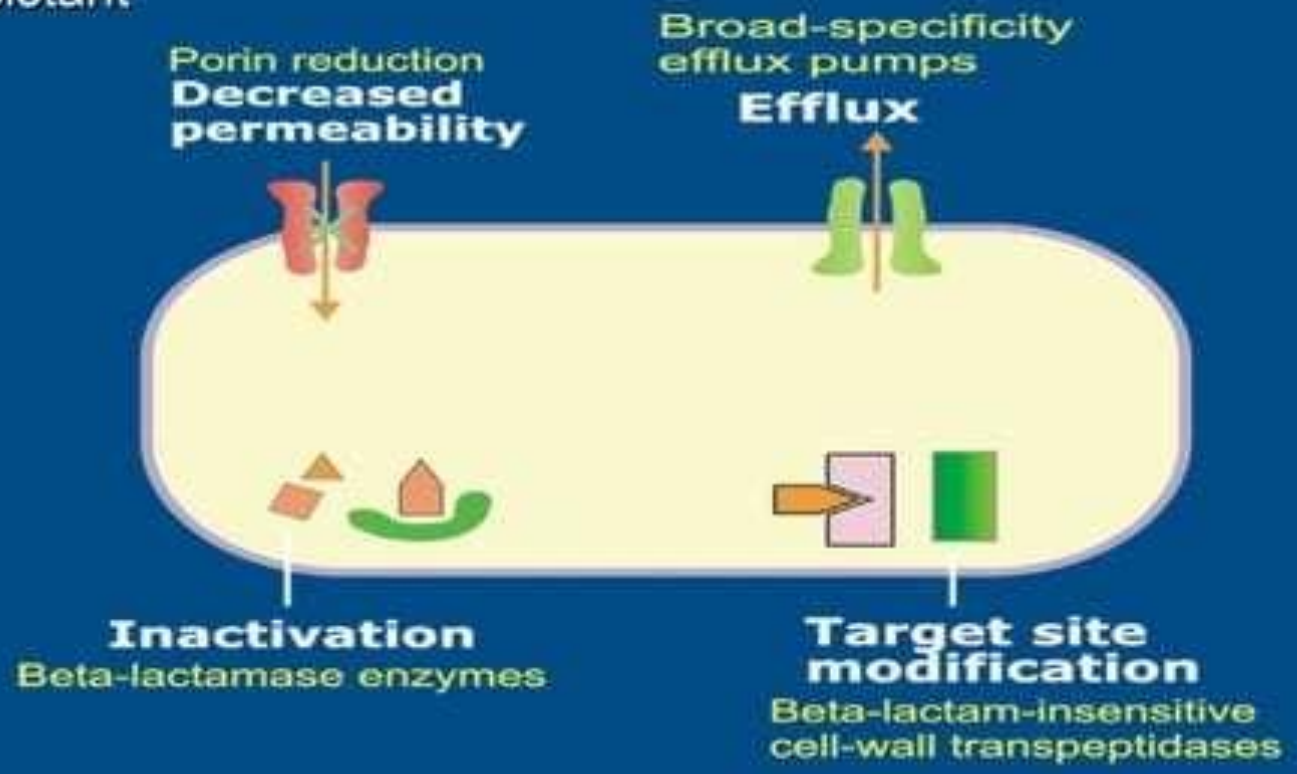


# Selection for antimicrobial-resistant Strains



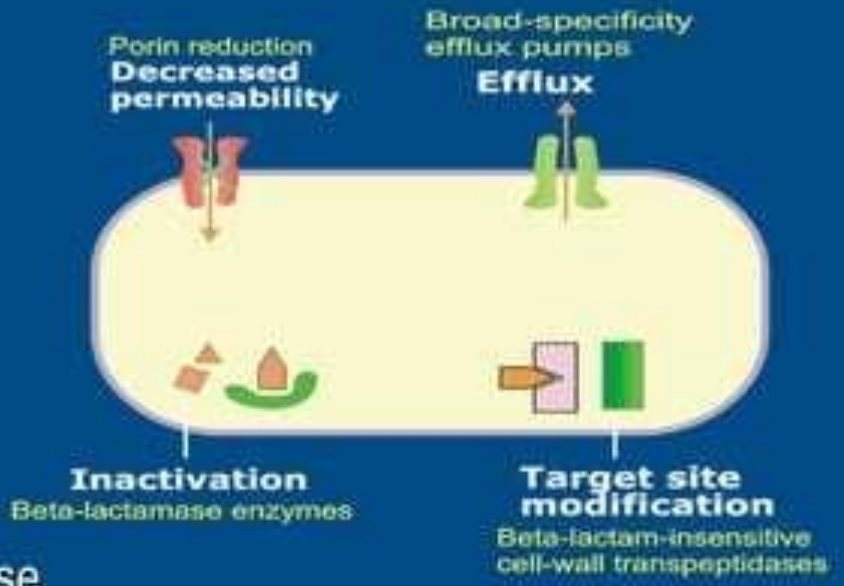
# Mechanisms of Resistance

- Antibiotics exert selective pressure that favours emergence of resistant organisms
- Bacteria employ several biochemical strategies to become resistant



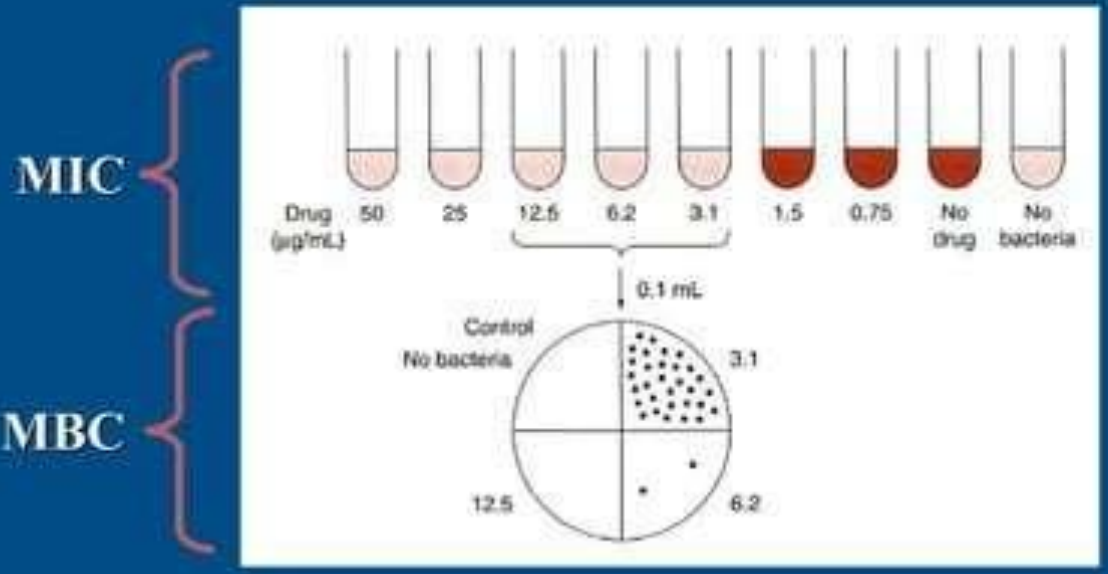
# Mechanisms of Resistance

- Altered influx
  - Gram negative bacteria
- Altered efflux
  - quinolones, tetracyclines
- Inactivation
  - β-lactamase
  - Chloramphenicol acetyl transferase
- Modification of target sites
  - Altered PBP (penicillins)
  - New PBP (MRSE, MRSA)
  - Modification in ribosomes (macrolide-resistant *S. pneumoniae*)
- Replacement of a sensitive pathway
  - Acquisition of a resistant enzyme (sulfonamides, trimethoprim)



# Antibiotic Susceptibility Testing (AST)

- Disk diffusion (Kirby Bauer)
- Serial dilution (Macro and micro)
- Antimicrobial gradient method (E test)




# Review

| | Mechanism of action | Main resistance mechanisms |
|---|---|---|
| **Beta-lactams** | Inactivate PBPs (peptidoglycan synthesis) | •Beta-lactamases<br>•Low affinity PBPs<br>•Decreased transport |
| **Glycopeptides** | Bind to precursor of peptidoglycan | • Modification of precursor |
| **Aminoglycosides** | Inhibit protein synthesis (bind to 30S subunit) | • Modifying enzymes (add adenyl or $PO_4$) |
| **Macrolides** | Inhibit protein synthesis (bind to 50S subunit) | • Methylation of rRNA<br>• Efflux pumps |
| **Quinolones** | Inhibit DNA gyrase (DNA synthesis) | • Altered target enzyme<br>• Efflux pumps |