Medical Bacteriology

Undergraduate students
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Antimicrobial Chemotherapy

Outlines

- Terminology
- Mechanisms of Action of Antimicrobials
- Inhibition of cell wall synthesis
- Inhibition of Protein Synthesis
- Disruption of cell membrane function
- Inhibition of Nucleic acid Synthesis
- Additional Drug Mechanisms
- Adverse Side Effects
- Resistance to Antimicrobial drugs
- Chemoprophylaxis
- Probiotics

- **Antimicrobial agents**, are a special group of chemotherapeutic agents used to treat diseases caused by microbes.
- **Antibiotic**—antimicrobials of microbial origin, most of which are produced by fungi or by bacteria of the genus *Streptomyces*.

- agents synthesized in the laboratory are called **synthetic drugs**.
- **semi-synthetic drugs**.

THE SPECTRUM OF ACTIVITY

- The range of different microbes against which an antimicrobial agent acts is called its **spectrum of activity**.
- **broad spectrum**
- **narrow spectrum**

- A broad-spectrum drug is especially useful a patient is seriously ill with an infection caused by an unidentified organism
- However, if the identity of the organism is known, a narrow spectrum drug should be used. Using such a drug minimizes the destruction of the host's *microflora*

TABLE 13.1 The Spectrum of Activity of Selected Antimicrobial Agents

Organisms Affected	Broad-Spectrum Agentsa*	Narrow-Spectrum Agents
Bacteroides and other anaerobes	Cephalosporins	Lincomycin Clindamycin
Yeasts	Chloramphenicol	Nystatin
Gram-positive bacteria	Gentamicin	Penicillin G
	Ampicillin	Erythromycin
Gram-negative bacteria	Kanamycin	Polymyxins
Streptococci and some Gram-negative bacteria	Tetracyclines	Streptomycin
Staphylococci and some clostridia	Tetracyclines	Vancomycin

*Broad-spectrum agents affect most bacteria.

ANTIMICROBIAL ACTION

- **bacteriostatic.** These drugs depend on the normal host defenses to kill or eliminate the pathogen after its growth has been inhibited.
- example, sulfa drugs, which are frequently prescribed for urinary tract infections, inhibit the growth of bacteria in the bladder until they are eliminated by the body's defenses. Drugs that kill bacteria are **bactericidal.**

Mechanisms of Antimicrobial Action

- Bacteria have their own enzymes for
  - Cell wall formation
  - Protein synthesis
  - DNA replication
  - RNA synthesis
  - Synthesis of essential metabolites

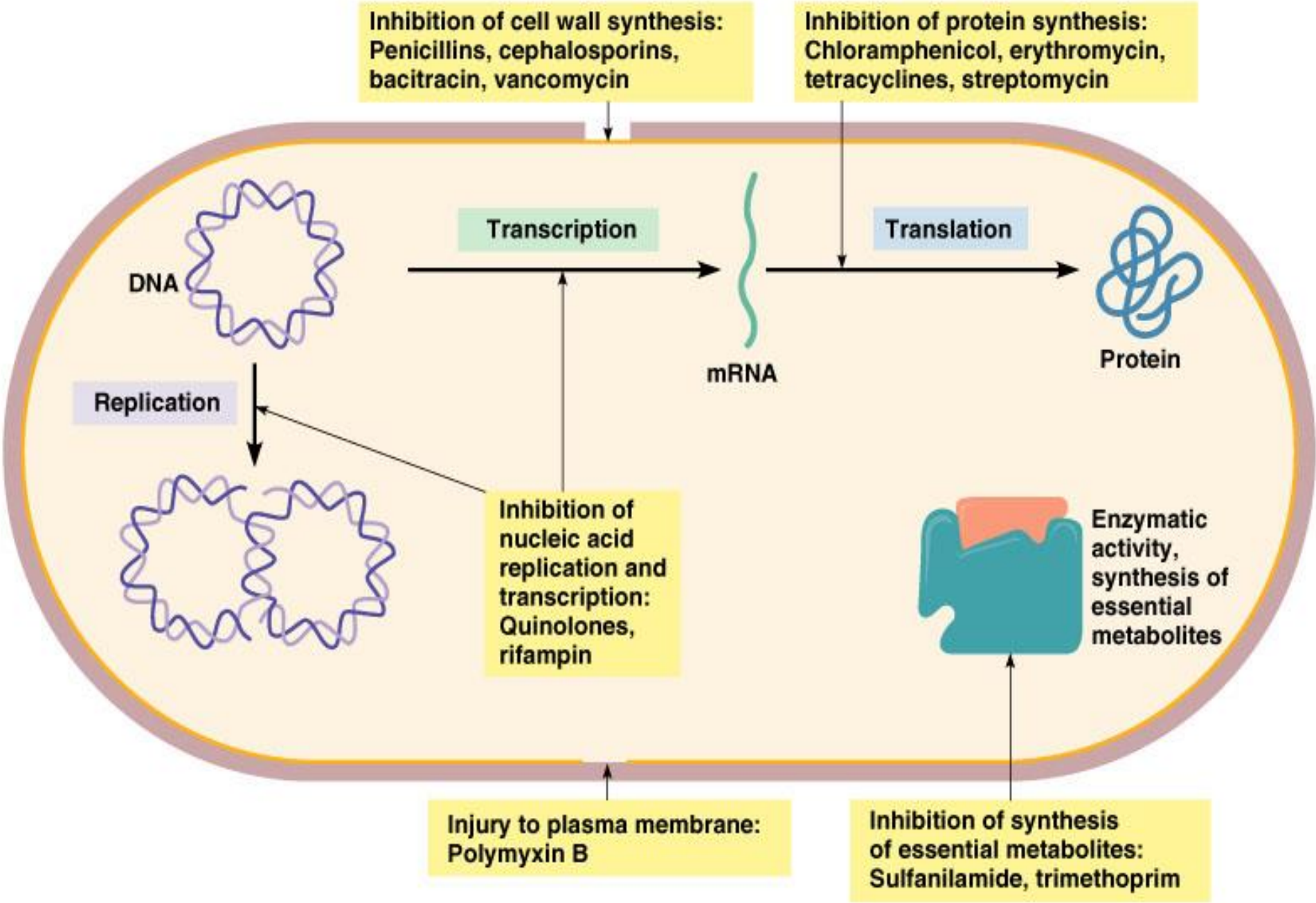
Differences between human cells Vs Bacterial Cells

(Makes the antibacterial selective)

- Human cells don't posses wall 1
  - (Peptidoglycans = peptides + sugar)
- Human cell membrane is different 2
  - (Sterol)
- Human cells take preformed dihydrofolic acid 3
  - (no need of PABA in human)
- Dihydrofolic acid reducatase enzyme is different 4
  - (thousand time affinity)
- Topoisomerase II are different 5
  - (in bacteria IV, DNA Gyrase)
- RNA polymerase is different 6
- Ribosome 60S subunit (in bacteria 50S) 7
- Ribosome 40S subunit (in bacteria 30S) 8

Mechanism of Action

- **Cell Wall synthesis inhibition-**
  - Beta-lactams, Vancomycin, Cycloserines(Pyrazinamide)
- **Cell membrane Leakage-**
  - **Polypeptides (polymyxin B,colisitin)**
- **Folate Synthesis inhibition-**
  - Sulfonamides, Cotrimaxazole
  - **DNA gyrase and Topoisomerase inhibition-**
  - Fluroquinolones
- **RNA polymerase inhibition-**
  - Rifampicin,,
- **Protein Synthesis Inhibition-**
  - Aminoglycosides, tetracyclines, Chloramphenicol, Macrolides, Clindamycin

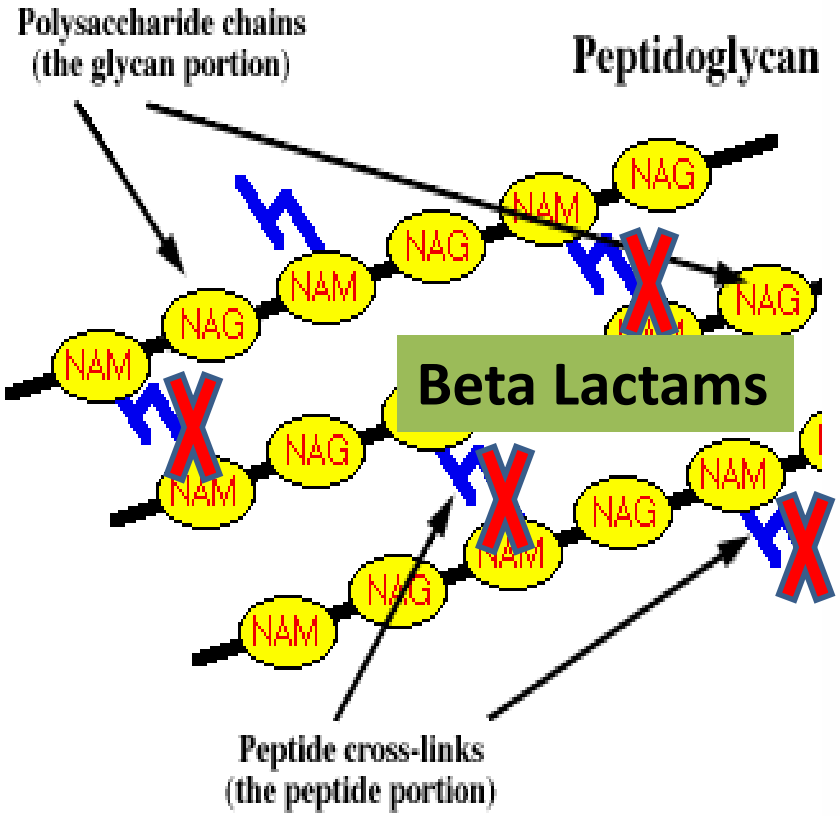


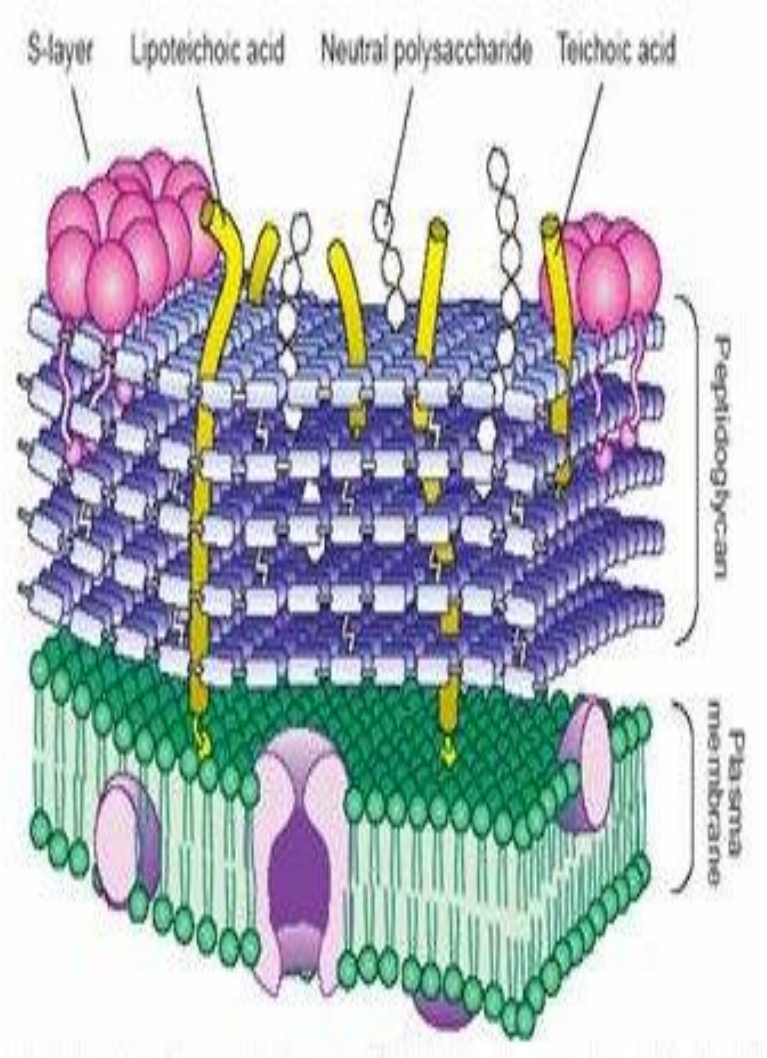
Inhibition of Cell Wall Synthesis

- The peptidoglycan : shape and rigidity.

- glycans *N*-acetylglucosamine and *N*-acetylmuramic acid
- Mature peptidoglycan is held together by cross-linking of short peptide side chains hanging off the long glycan molecules.
- This cross-linking process is the target of two of the most important groups of antimicrobics, the β-lactams and the glycopeptides (vancomycin and teicoplanin).

Cell Wall

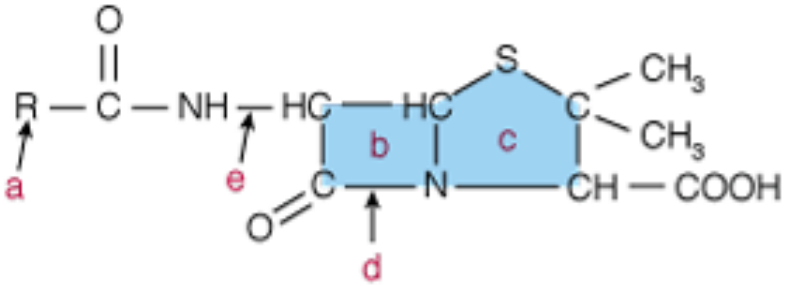

Polysaccharide chains
(the glycan portion)
Peptidoglycan
NAG
NAM
Beta Lactams
Peptide cross-links
(the peptide portion)


S-layer
Lipoteichoic acid
Neutral polysaccharide
Teichoic acid
Peptidoglycan
Plasma membrane

β-Lactam Antimicrobials

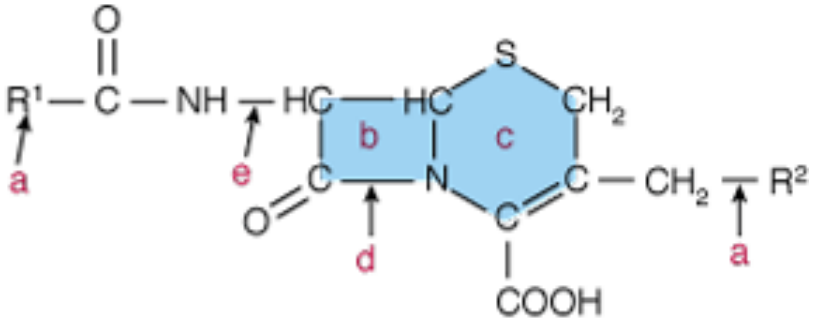
- The β-lactam antimicrobial agents include the penicillins, cephalosporins, carbapenems, and monobactams.
- Their name derives from the presence of a β-lactam ring in their structure; this ring is essential for antibacterial activity.
- The β-lactam antibacterial agents interfere with the transpeptidation reactions that seal the peptide cross-links between glycan chains..
- These targets of all the β-lactams are commonly called penicillin-binding proteins (PBPs).

Penicillins



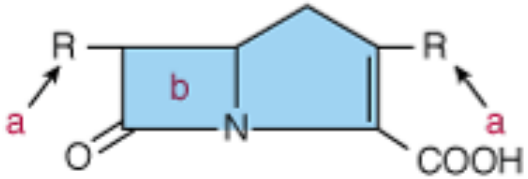
6-Aminopenicillanic acid

Cephalosporins

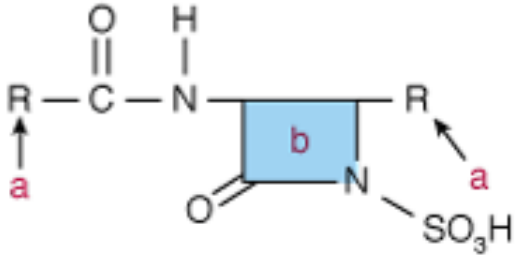


7-Aminocephalosporanic acid

Carbapenems



Monobactams



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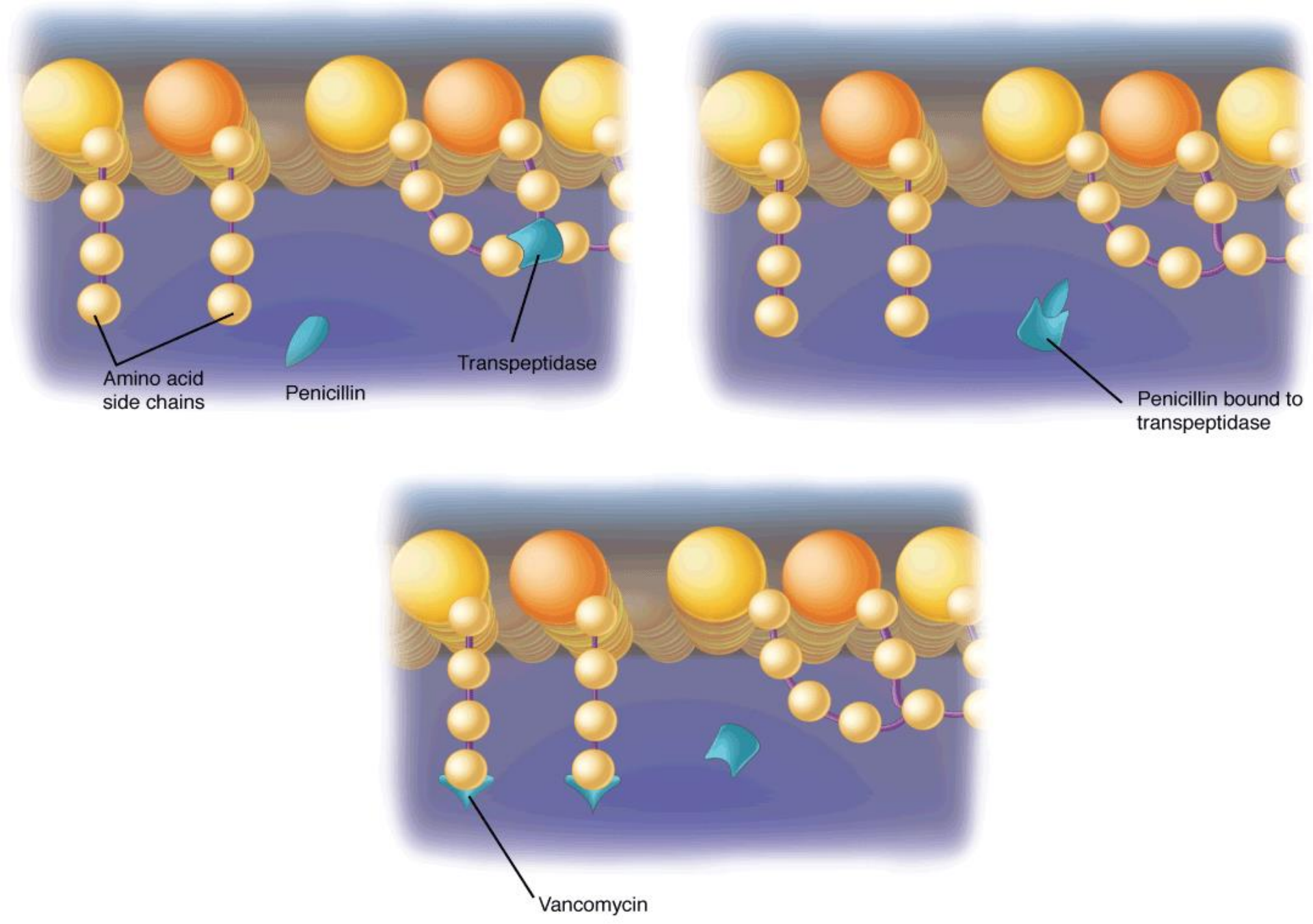

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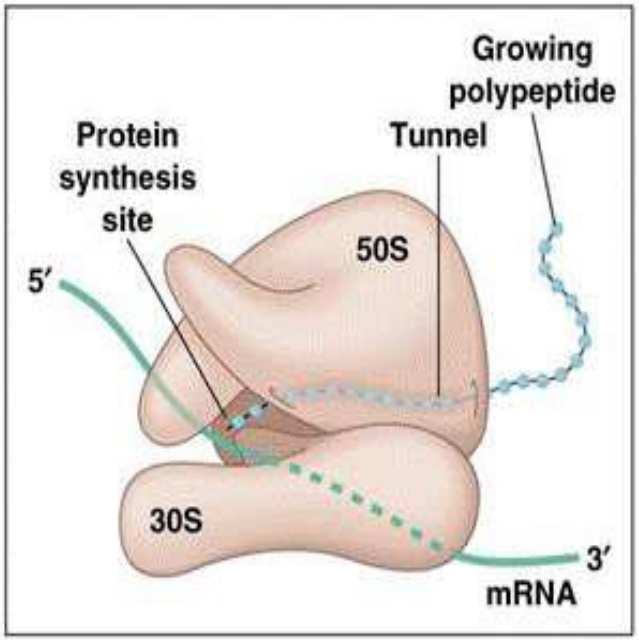
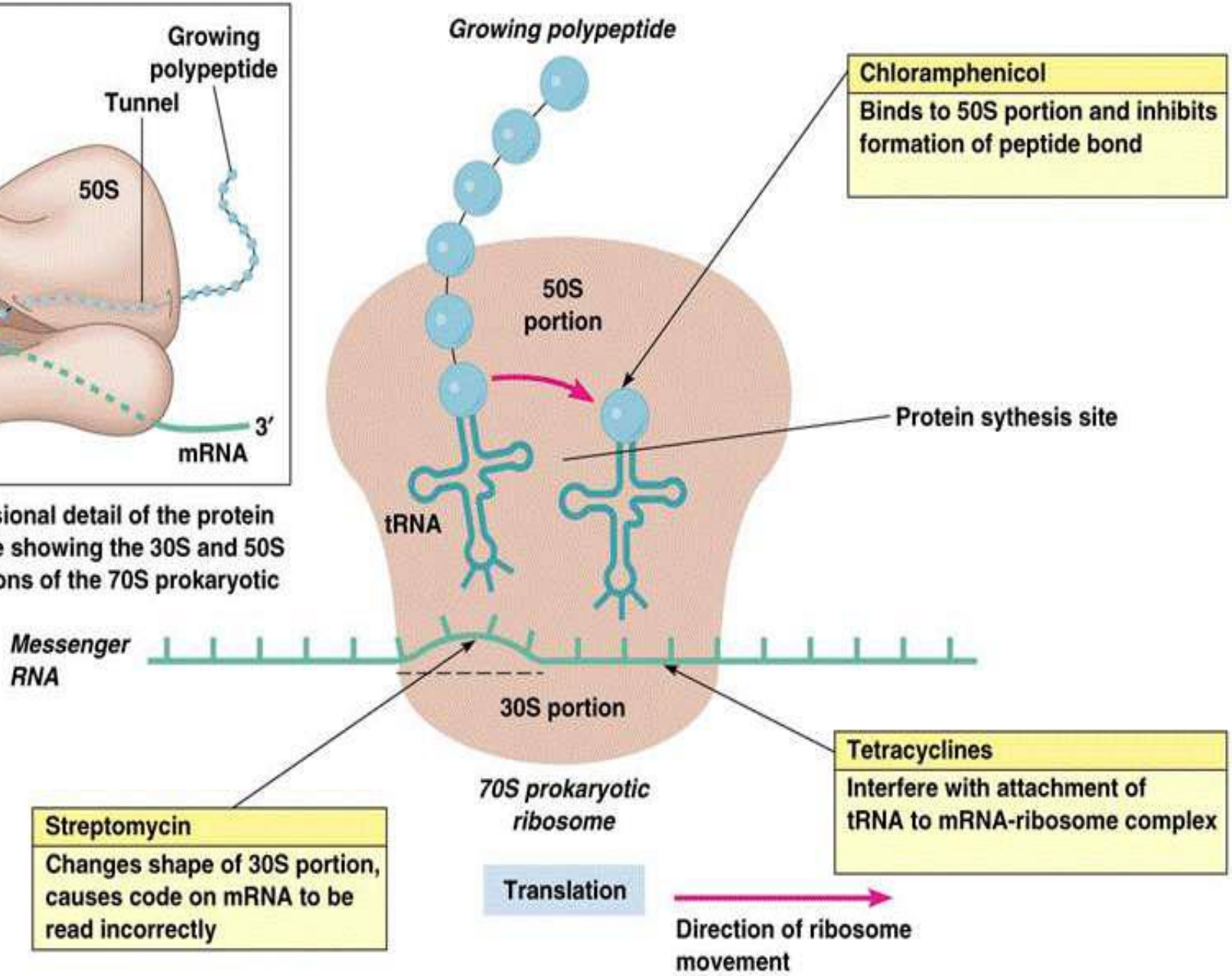
Inhibition of Protein Synthesis

- Several drugs inhibit protein synthesis in bacteria without significantly interfering with protein synthesis in human cells.
- This selectivity is due to the differences between bacterial and human ribosomal proteins, RNAs, and associated enzymes.
- Bacteria have 70S ribosomes with 50S and 30S subunits, whereas human cells have 80S ribosomes with 60S and 40S subunits.



(a) Three-dimensional detail of the protein synthesis site showing the 30S and 50S subunit portions of the 70S prokaryotic ribosome.



(b) In the diagram the black arrows indicate the different points at which chloramphenicol, the tetracyclines, and streptomycin exert their activities.

Antibiotic	Ribosomal Subunit	Mode of Action	Bactericidal or Bacteriostatic
Aminoglycosides	30S	Blocks functioning of initiation complex and causes misreading of mRNA	Bactericidal
Tetracyclines	30S	Blocks tRNA binding to ribosome	Bacteriostatic
Chloramphenicol	50S	Blocks peptidyltransferase	Both
Erythromycin	50S	Blocks translocation	Primarily bacteriostatic
Clindamycin	50S	Blocks peptide bond formation	Primarily bacteriostatic

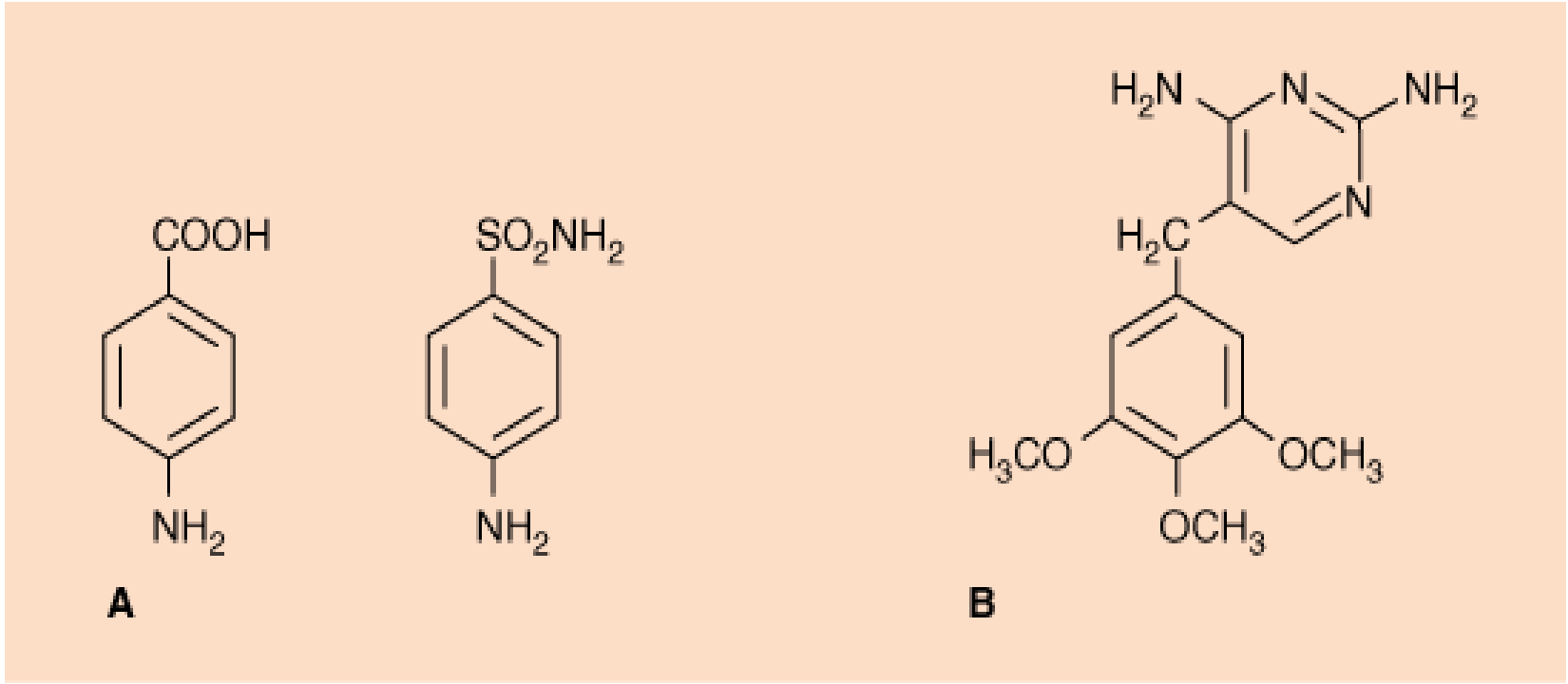
Alteration of Cell Membrane Function

- **There are few antimicrobial compounds that act on the cell membrane because the structural and chemical similarities of bacterial and human cell membranes make it difficult to provide sufficient selective toxicity.**
- **Polymyxins are a family of polypeptide antibiotics of which the clinically most useful compound is polymyxin E (colistin). It is active against gram-negative rods, especially *P. aeruginosa.***

- **Daptomycin is a cyclic lipopeptide that disrupts the cell membranes of gram-positive cocci. It is bactericidal for organisms such as *S. aureus, S. epidermidis, Str. pyogenes, Ent. faecalis,* and *Ent. Faecium.***

Inhibition of Nucleic Acid Synthesis

- **Sulfonamides** and **trimethoprim** inhibit the **synthesis of tetrahydrofolic acid—**the main donor of the methyl groups that are required to synthesize adenine, guanine, and thymine.
- **Sulfonamides** are structural analogues of *p*-aminobenzoic acid, which is a component of folic acid.
- **Trimethoprim** inhibits **dihydrofolate reductase—**the enzyme that reduces dihydrofolic acid to tetrahydrofolic acid.
- A combination of sulfamethoxazole and trimethoprim is often used because bacteria resistant to one drug will be inhibited by the other.

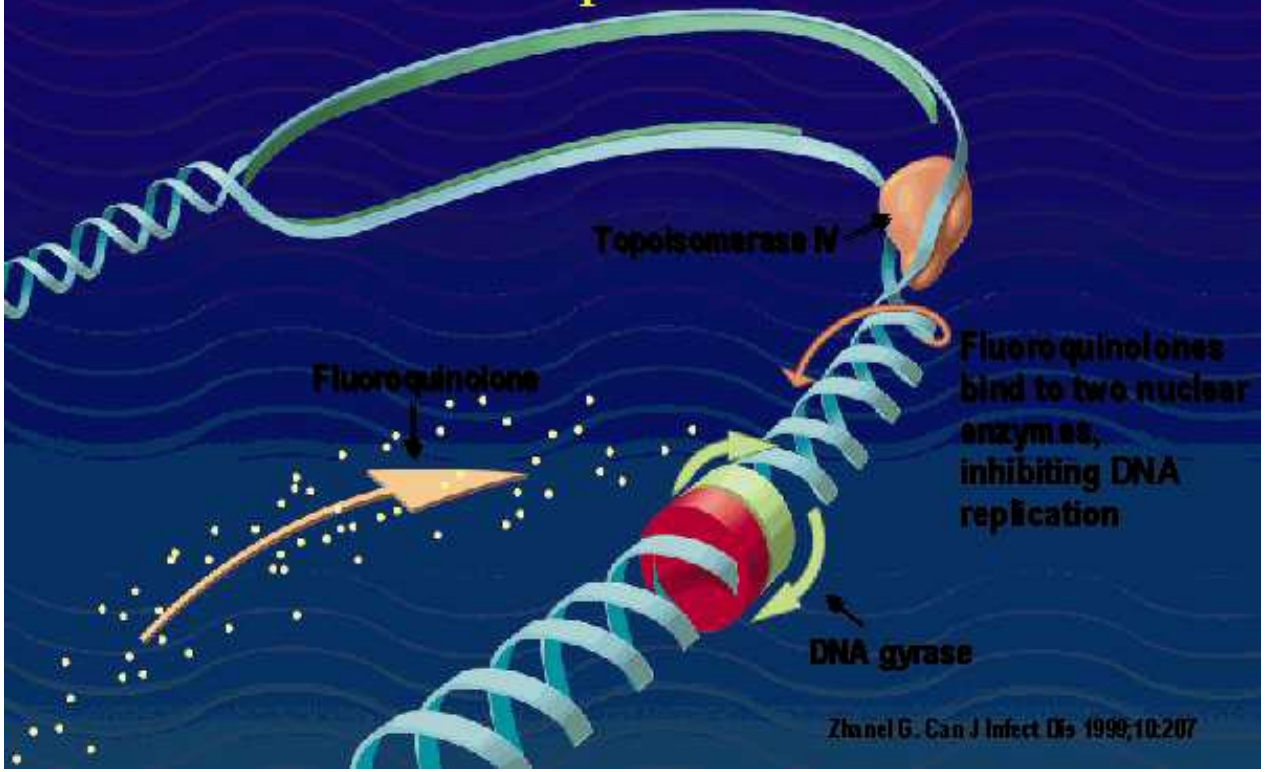


Para-aminobenzoic acid (PABA) + Other components → Dihydropteroate synthase (Inhibited by sulfonamide) → Dihydrofolic acid (DHF) → Dihydrofolate reductase (Inhibited by trimethoprim) → Tetrahydrofolic acid (THF)

C

- **Quinolones** inhibit DNA synthesis in bacteria by **blocking DNA gyrase** (topoisomerase)—the enzyme that unwinds DNA strands so that they can be replicated.
- Quinolones are a family of drugs that includes ciprofloxacin, ofloxacin, and levofloxacin.

Mechanism of Action of Fluoroquinolones



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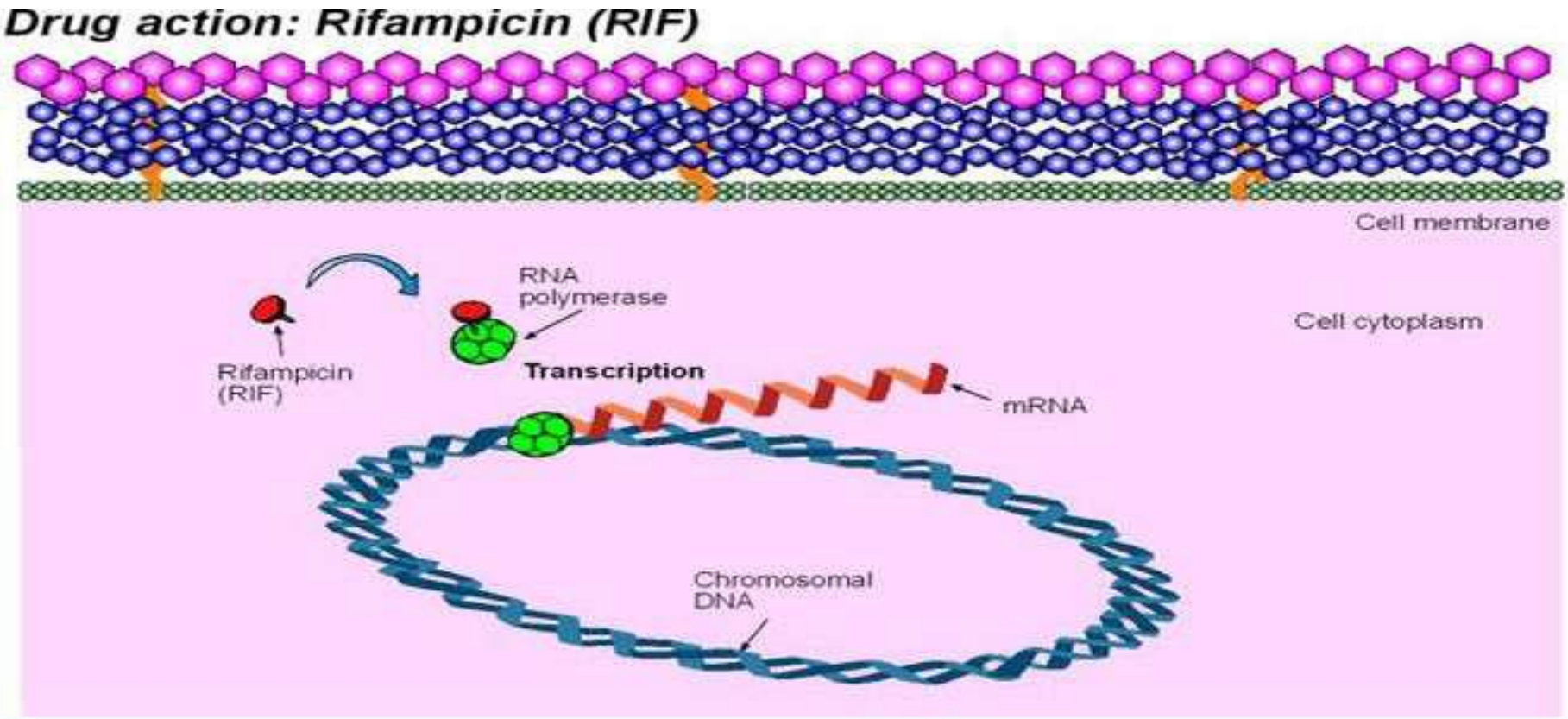
Rifampin inhibits RNA synthesis in bacteria by **blocking the RNA polymerase** that synthesizes mRNA.



The first-line antibiotic drug rifampicin (RIF) interferes with RNA transcription in Mycobacterium tuberculosis. RIF binds to the β-subunit of the DNA-dependent RNA polymerase enzyme complex and inhibits transcription of messenger RNA (mRNA). The mRNA transcripts are essential requirements for protein synthesis (translation).

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ADDITIONAL DRUG MECHANISMS

- **Isoniazid inhibits the synthesis of mycolic acid** a long-chain fatty acid found in the cell wall of mycobacteria.

- **Metronidazole** is **effective against anaerobic bacteria and certain protozoa** because it **acts as an electron sink**, taking away the electrons that the organisms need to survive.

ADVERSE SIDE EFFECTS

- The side effects of antimicrobial agents on infected persons (hosts) fall into three general categories:

(1) toxicity,

(2) allergy, and

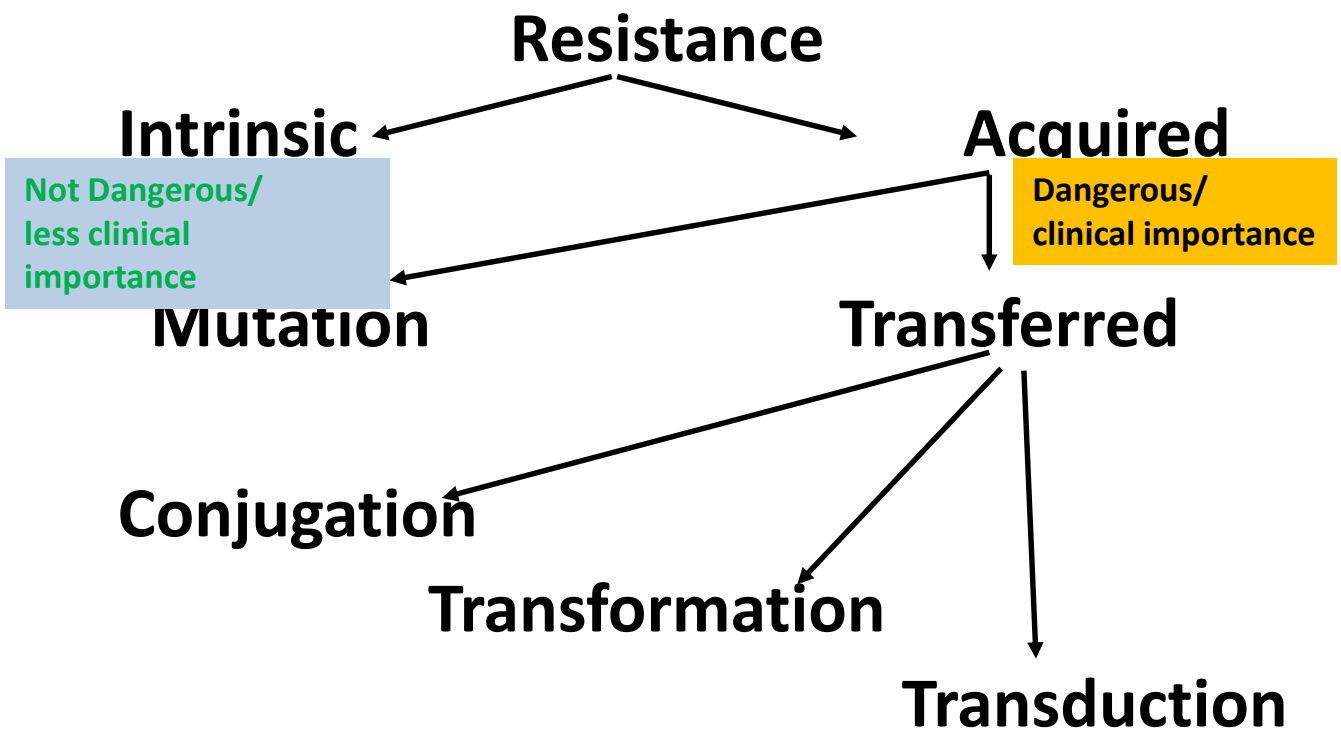
(3) disruption of normal microflora.

- **The development of resistance to antibiotics can also be thought of as a side effect on the microorganisms.**

RESISTANCE TO ANTIMICROBIALS

- In some cases, certain types of bacteria are inherently resistant to the effects of a particular drug; this is called **innate,** or **intrinsic, resistance.**
- Members of the genus *Mycoplasma* lack a cell wall, penicillin&drugs that…….
- Many Gram-negative organisms are intrinsically resistant to certain drugs because the lipid bilayer of their outer membrane excludes entry of the drug.
- **acquired resistance.**

Mechanisms Of Resistance

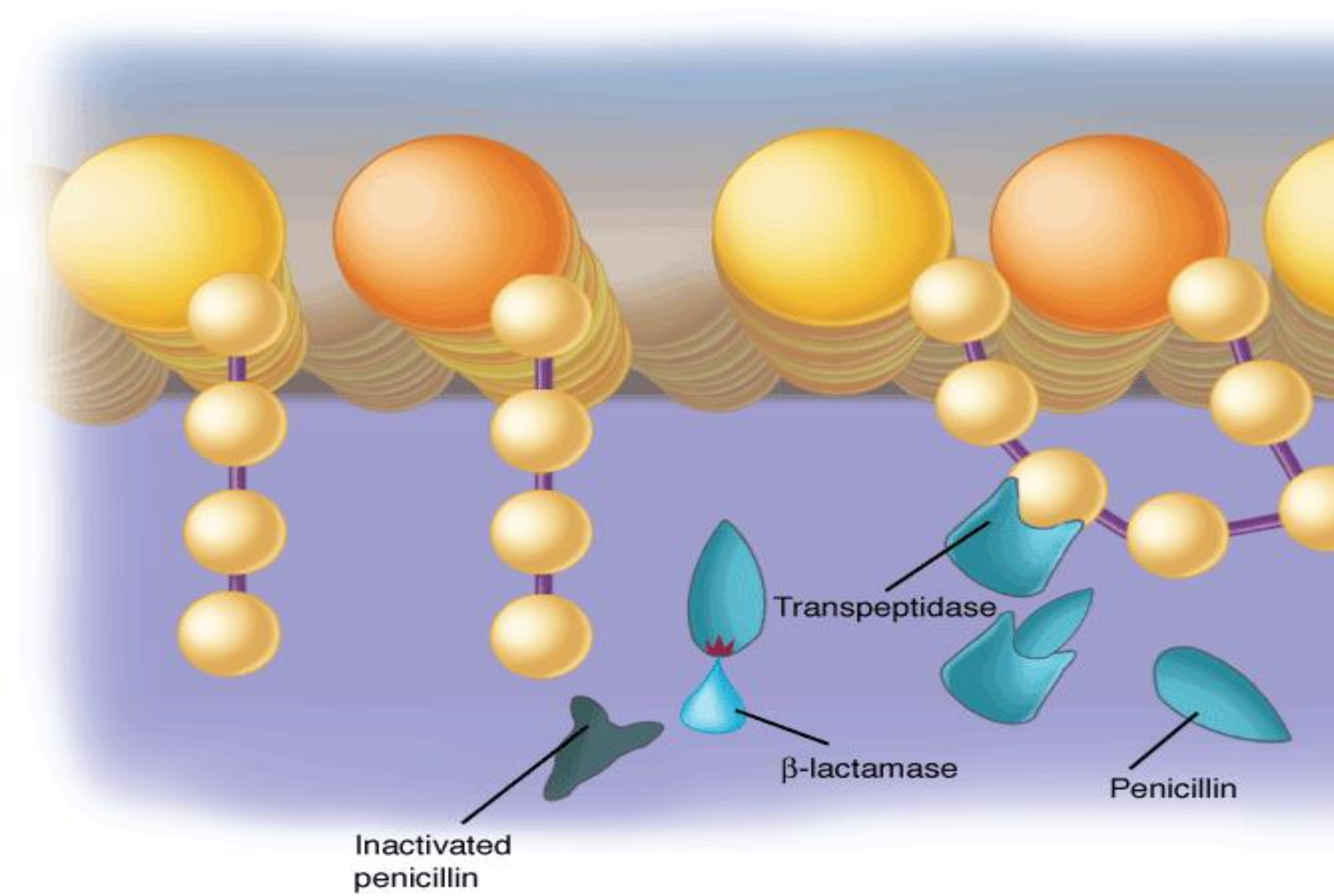


Mechanisms of Resistance

1. Microorganisms produce enzymes that destroy the active drug. ***Examples:*** Staphylococci resistant to penicillin G produce a β-lactamase that destroys the drug.

Other β-lactamases are produced by Gram-negative rods.

- Gram-negative bacteria resistant to aminoglycosides (by virtue of a plasmid) produce enzymes that destroy the drug.



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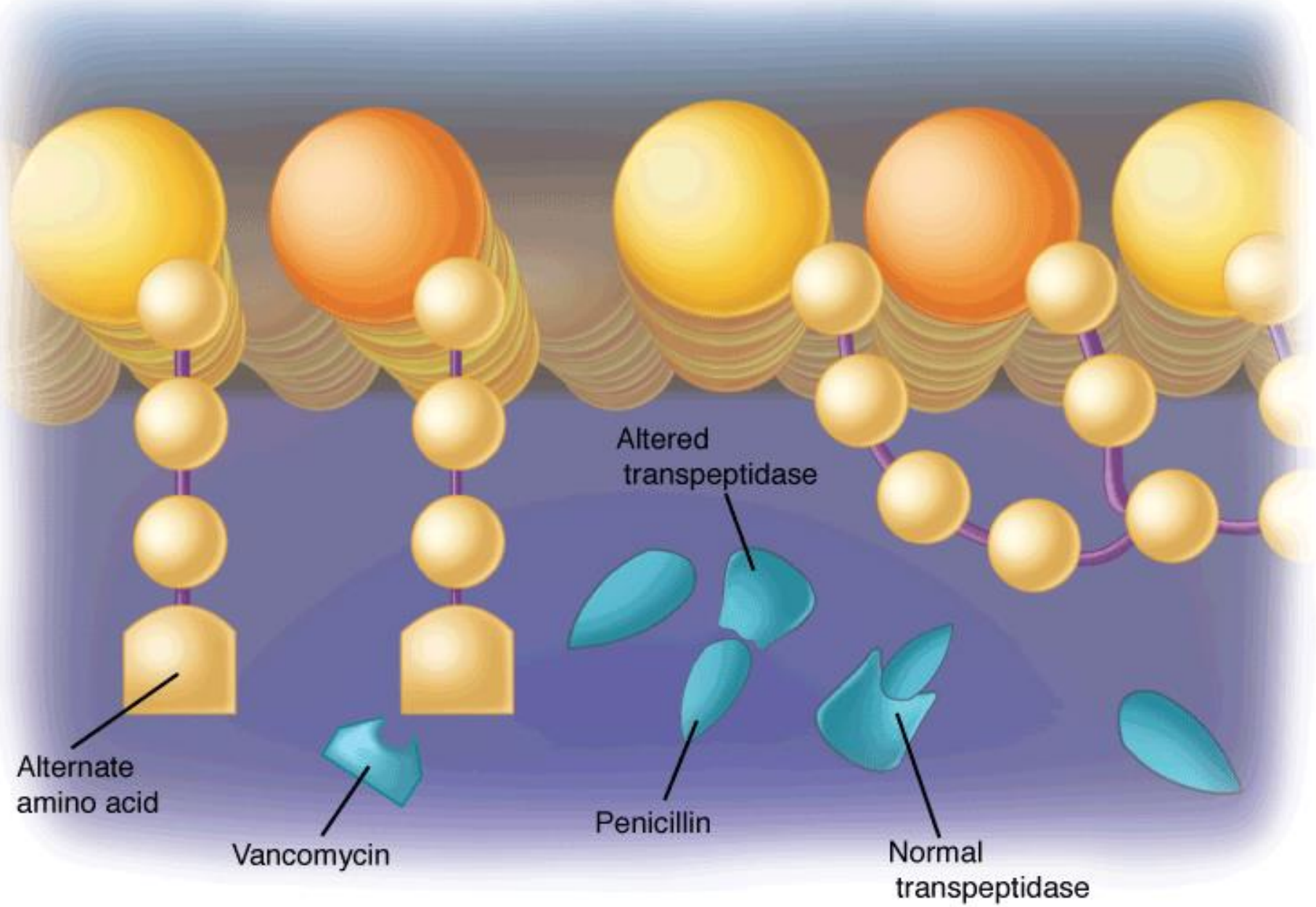
Changing Permeability

- **2.** Microorganisms change their permeability to the drug. ***Examples:*** **Tetracyclines** accumulate in susceptible bacteria but not in resistant bacteria.
- **Resistance to polymyxins** is also associated with a change in permeability to the drugs.
- **Streptococci have a natural permeability barrier to aminoglycosides**. This can be partly overcome by the simultaneous presence of a cell wall-active drug such as a penicillin.
- Resistance to amikacin and to some other aminoglycosides may depend on a lack of permeability to the drugs caused by an outer membrane change that impairs active transport into the cell.

Alteration of receptors

- **3.** Microorganisms develop an altered structural target for the drug. ***Examples:*** Erythromycin-resistant organisms have an **altered receptor on the 50S subunit of the ribosome**,
- Resistance to some penicillins and cephalosporins may be a function of the loss or **alteration of PBPs.**
- **Eg.Penicillin resistance in *Streptococcus pneumoniae* and enterococci** is attributable to **altered PBPs**.



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Alteration in Metabolic Pathway

4. Microorganisms develop an altered metabolic pathway that bypasses the reaction inhibited by the drug.

Example: Some **sulfonamide-resistant** bacteria do not require extracellular PABA but, similar to mammalian cells, can use preformed folic acid.

Developing efflux pumps

- **5.** Microorganisms can **develop efflux pumps** that transport the antibiotics out of the cell.
- Many Gram-positive and especially Gram-negative organisms have developed this mechanism for tetracyclines (common), macrolides, fluoroquinolones, and even β-lactam agents.

Origin of Antimicrobial Resistance

A-Genetic Basis of Resistance

1-Chromosome-Mediated Resistance

- **Chromosomal resistance is due to a mutation in the gene that codes for either the target of the drug or the transport system in the membrane that controls the uptake of the drug.**
- **The frequency of spontaneous mutations is much lower than the frequency of acquisition of resistance plasmids.**
- **Therefore, chromosomal resistance is less of a clinical problem than is plasmid-mediated resistance.**

2-Plasmid-Mediated Resistance

Plasmid-mediated resistance is very important from a clinical point of view for three reasons:

- **It occurs in many different species, especially gram-negative rods.**
- **Plasmids frequently mediate resistance to multiple drugs.**
- **Plasmids have a high rate of transfer from one cell to another, usually by conjugation.**

Resistance plasmids (resistance factors, R factors) are extrachromosomal, circular, double-stranded DNA molecules that carry the genes for a variety of enzymes that can degrade antibiotics and modify membrane transport systems.

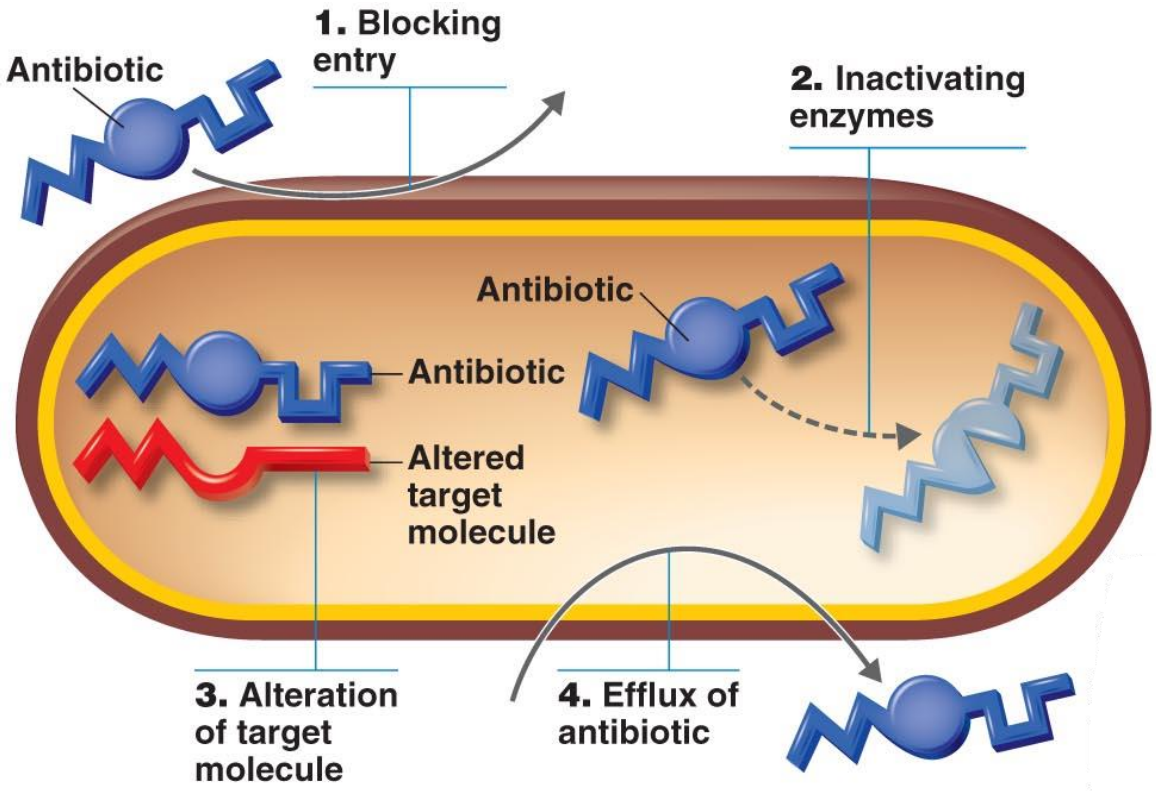
3-Transposon-Mediated Resistance

- **Transposons are genes that are transferred either within or between larger pieces of DNA such as the bacterial chromosome and plasmids.**
- **A typical drug resistance transposon is composed of three genes, the genes code for (1) transposase, the enzyme that catalyzes excision and reintegration of the transposon, (2) a repressor that regulates synthesis of the transposase, and (3) the drug resistance gene.**

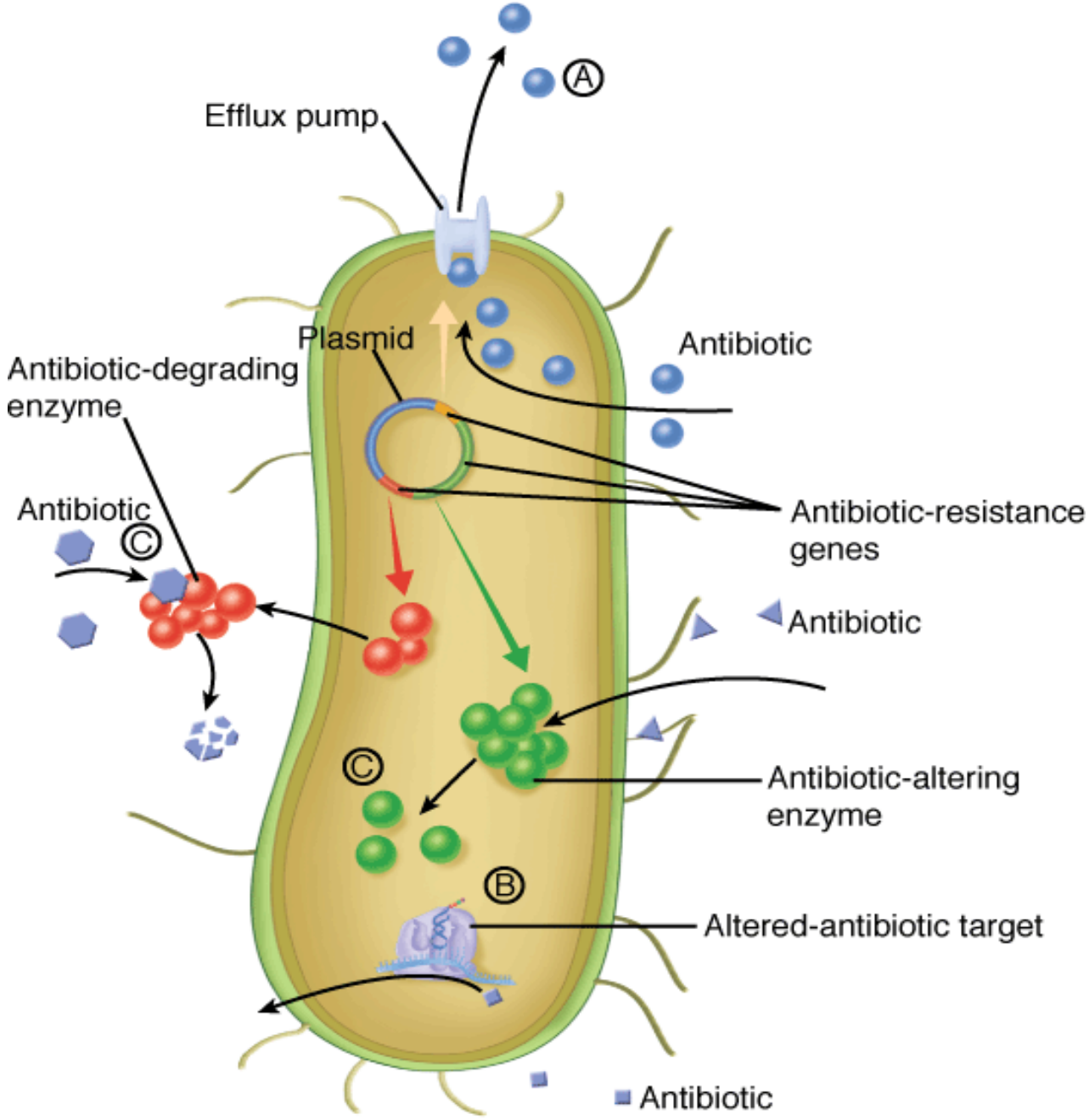
B-Non-genetic Basis of Resistance

- **Bacteria can be in a resting state, i.e., not growing; they are therefore insensitive to cell wall inhibitors such as penicillins and cephalosporins.**
- **Similarly, *M. tuberculosis* can remain dormant in tissues for many years, during which time it is insensitive to drugs.**
- **Under certain circumstances, organisms that would ordinarily be killed by penicillin can lose their cell walls, survive as protoplasts, and be insensitive to cell wall–active drugs.**

Mechanisms of Antibiotic Resistance



Most resistance are on plasmid



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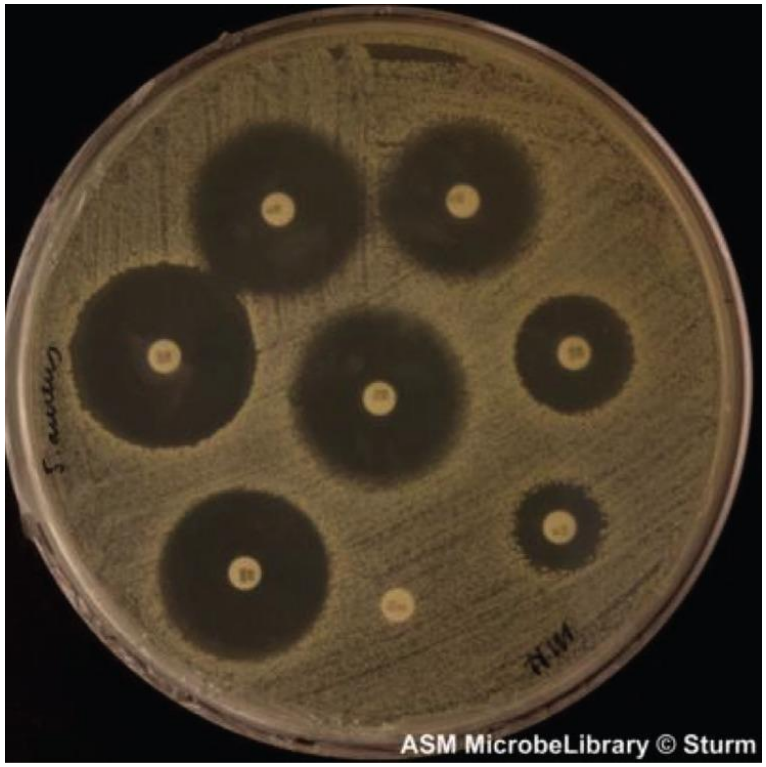
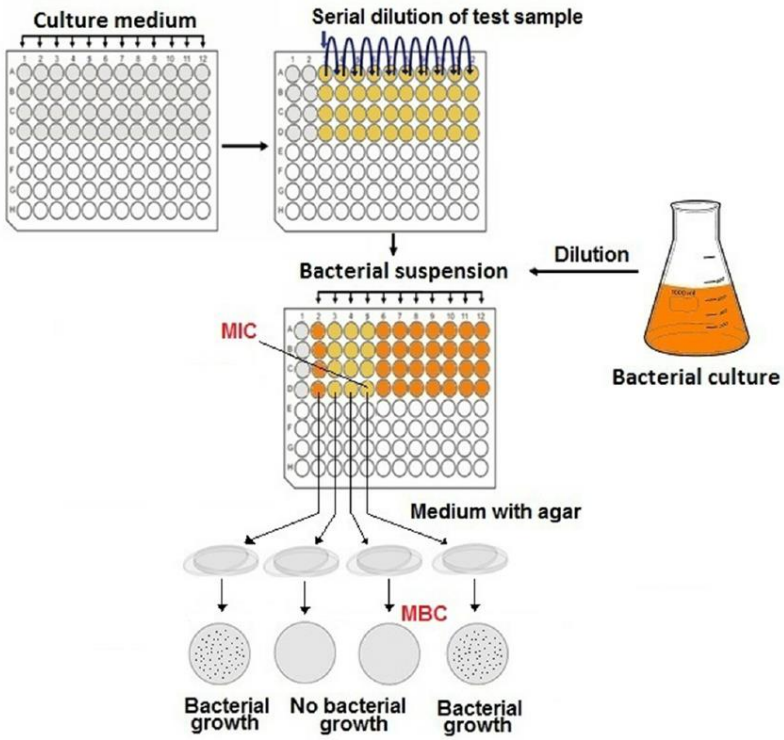
Determining the Susceptibility of a Bacterial Strain to an Antimicrobial Drug

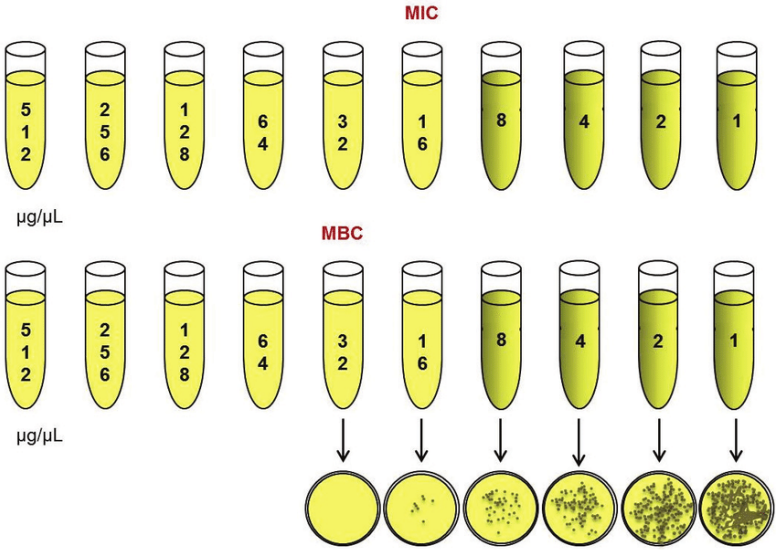
- **Kirby-Bauer disk diffusion Method**
- **Minimum Inhibitory Concentration (MIC)**

Kirby-Bauer disk diffusion Method



Minimam Inhibitory Concentration





CHEMOPROPHYLAXIS

- Chemoprophylactic drugs are given primarily in three circumstances:
- to prevent surgical wound infections,
- to prevent opportunistic infections in immunocompromised patients,
- and to prevent infections in those known to be exposed to pathogens that cause serious infectious diseases.

PROBIOTICS

- are live, non pathogenic bacteria that may be effective in the treatment or prevention of certain human diseases.
- beneficial effect
- providing colonization resistance by which the non pathogen excludes the pathogen from binding sites on the mucosa,
- in enhancing the immune response against the pathogen, or in reducing the inflammatory response against the pathogen.
- For example, the oral administration of live *Lactobacillus rhamnosus* strain GG significantly reduces the number of cases of nosocomial diarrhea in young children.

Superbugs
(Microorganisms with multiple resistance)

- **MRSA** - Methicillin-resistant *Staphylococcus aureus*
- **VISA** - Vancomycin intermediate resistant Staphylococci
- **VRE** - Vancomycin-resistant enterococci
- **ESBLs** - Extended-spectrum beta-lactamases (microorganisms – resistant to cephalosporins and monobactams)
- **PRSP** - Penicillin-resistant *Streptococcus pneumoniae*
- **MRPA (MDR-PA)-** Multidrug resistant *Pseudomonas aeruginosa*
- **MRAB (MDR-AB)** - Multidrug resistant *Acinetobacter baumannii*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- **Q**- Which one of the following groups of antimicrobial agents acts on microorganisms by inhibiting protein synthesis?

(A) Fluoroquinolones

(B) Aminoglycosides

(C) Penicillins

(D) Glycopeptides (eg, vancomycin)

(E) Polymyxins

Next Lecture

- Staphylococci