Chemotherapy of Bacterial Infections

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Antibiotics
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Definitions of Antibiotics

- **OLD:** An antibiotic is a chemical substance produced by various species of microorganisms that is capable in small concentrations of inhibiting the growth of other microorganisms

- **NEW:** An antibiotic is a product produced by a microorganism **or a similar substance produced wholly or partially by chemical synthesis,** which in low concentrations, inhibits the growth of other microorganisms

Impact of Modern Healthcare on Life Expectancy

History

Paul Ehrlich

"Magic Bullet"

✔ **Chemicals with selective toxicity**

ORIGIN: Selective Stains

DRUG: Arsphenamine (1910)

"606" Salvarsan

NOBEL: 1908

History (cont'd)

Gerhard Domagk

- ✔ Drugs are changed in the body

ORIGIN: Prontosil
(Only active *in vivo*)

DRUG: Sulfanilamide (1935)

NOBEL: 1939

History (cont'd)

Alexander Fleming

- ✔ Microbes make antibiotics

ORIGIN: moldy culture plate

DRUG: Penicillin (1928)

NOBEL: 1945

History

(cont'd)

Selman Waksman

- ✔ Soil Streptomyces make antibiotics
- ✔ comes up with definition of antibiotic

ORIGIN: Penicillin development

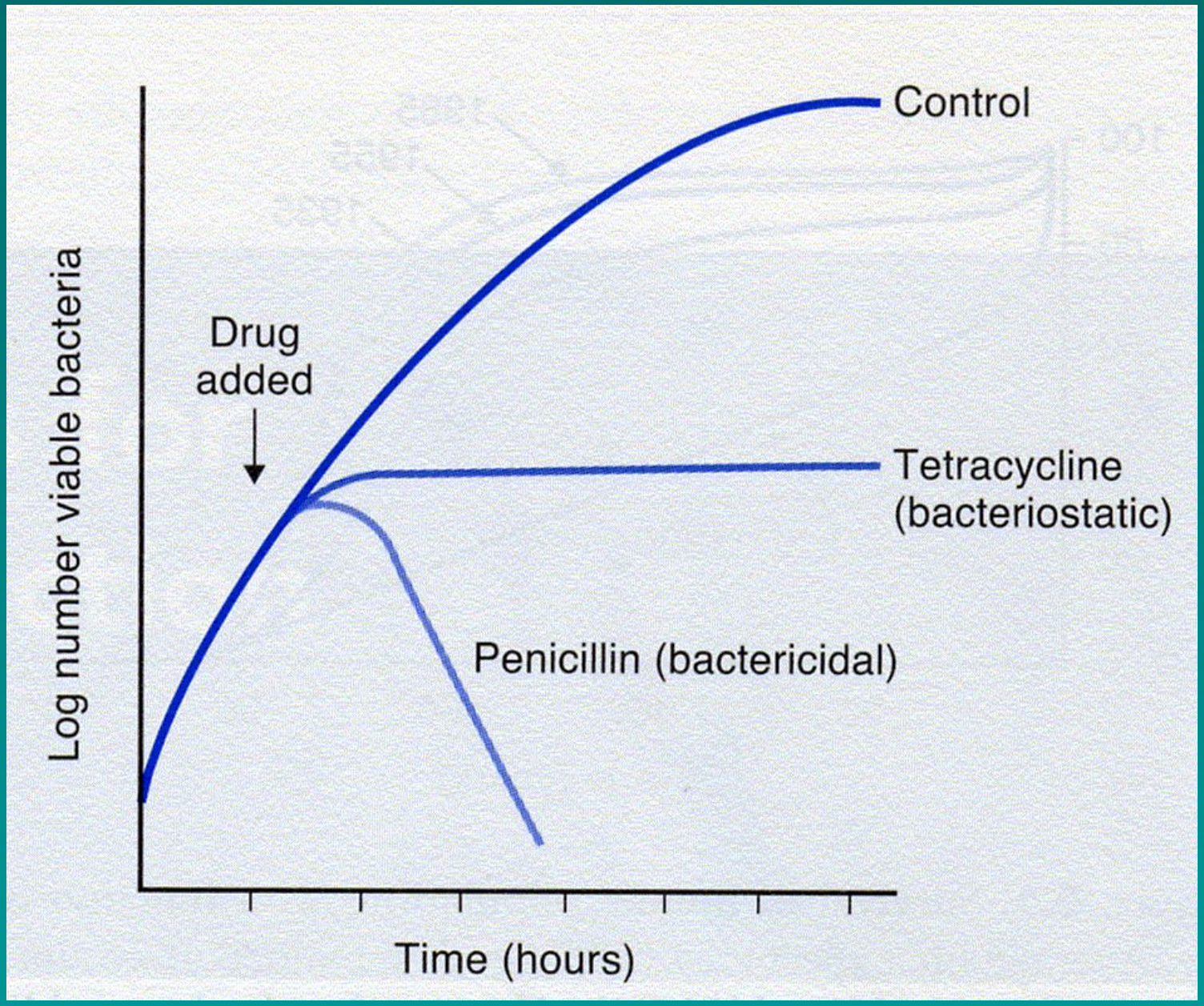
DRUG: Streptomycin (1943)

NOBEL: 1952

The Ideal Drug*

1. **Selective toxicity:** against target pathogen but not against host
   - ✔ LD_{50} (high) vs. MIC and/or MBC (low)
2. Bactericidal vs. bacteriostatic
3. **Favorable pharmacokinetics:** reach target site in body with effective concentration
4. **Spectrum of activity:** broad vs. narrow
5. Lack of "side effects"
   - ✔ Therapeutic index: effective to toxic dose ratio
6. Little resistance development

* There is no perfect drug.


Control
Drug
added
Tetracycline
(bacteriostatic)
Penicillin (bactericidal)
Log number viable bacteria
Time (hours)

Antibacterial spectrum—Range of activity of an antimicrobial against bacteria. A broad-spectrum antibacterial drug can inhibit a wide variety of gram-positive and gram-negative bacteria, whereas a **narrow-spectrum** drug is active only against a limited variety of bacteria.

Bacteriostatic activity—-The level of antimicro-bial activity that inhibits the growth of an organism. This is determined in vitro by testing a standardized concentration of organisms against a series of antimicrobial dilutions. The lowest concentration that inhibits the growth of the organism is referred to as the **minimum inhibitory concentration (MIC).**

Bactericidal activity—The level of antimicrobial activity that kills the test organism. This is determined in vitro by exposing a standardized concentration of organisms to a series of antimicrobial dilutions. The lowest concentration that kills 99.9% of the population is referred to as the **minimum bactericidal concentration (MBC).**

Antibiotic combinations—Combinations of antibiotics that may be used (1) to broaden the antibacterial spectrum for empiric therapy or the treatment of polymicrobial infections, (2) to prevent the emergence of resistant organisms during therapy, and (3) to achieve a synergistic killing effect.

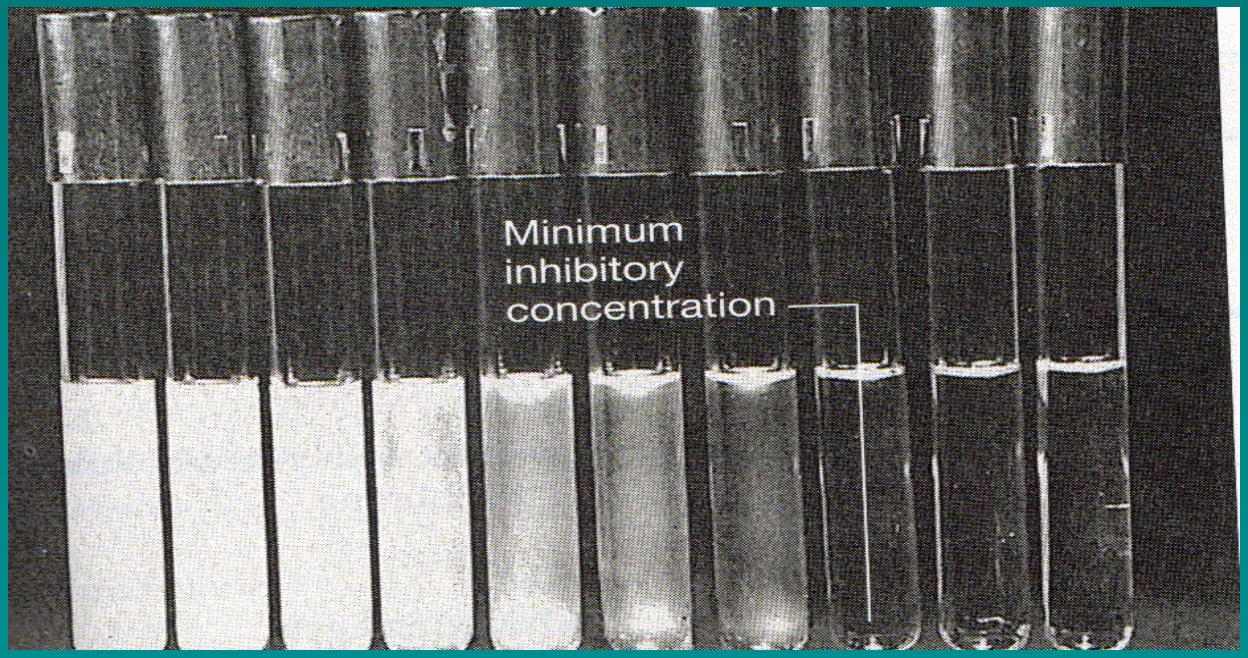
Antibiotic synergism—Combinations of two antibiotics that have enhanced bactericidal activity when tested together compared with the activity of each antibiotic.

Antibiotic antagonism—Combination of antibiotics in which the activity of one antibiotic interferes With the activity of the other (e.g., the sum of the activity is less than the activity of the individual drugs).

Beta-lactamase—An enzyme that hydrolyzes the beta-lactam ring in the beta-lactam class of antibiotics, thus inactivating the antibiotic. The enzymes specific for penicillins and cephalosporins aret he penicillinases and **cephalosporinases,** respectively.

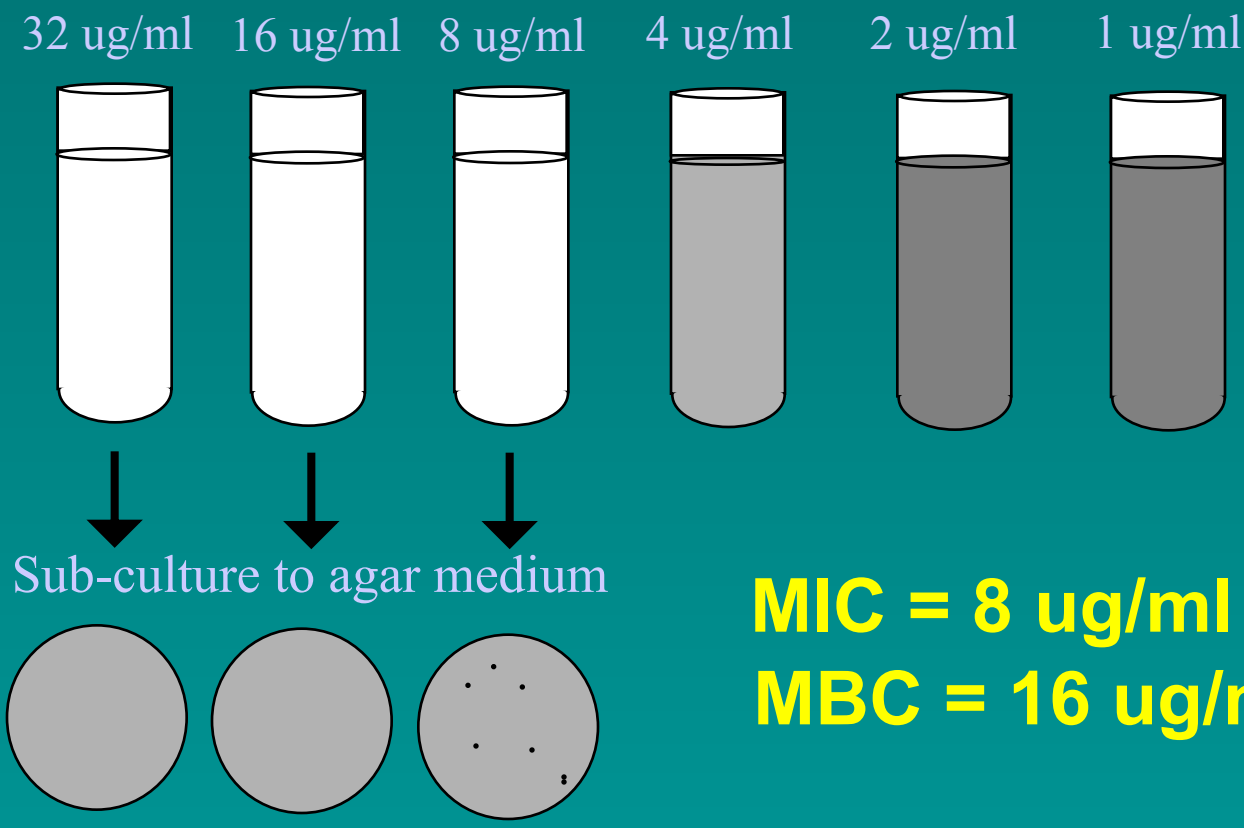
Susceptibility Tests

1. Broth dilution - MIC test



2. Agar dilution - MIC test

Minimal Inhibitory Concentration (MIC)
vs.
Minimal Bactericidal Concentration (MBC)



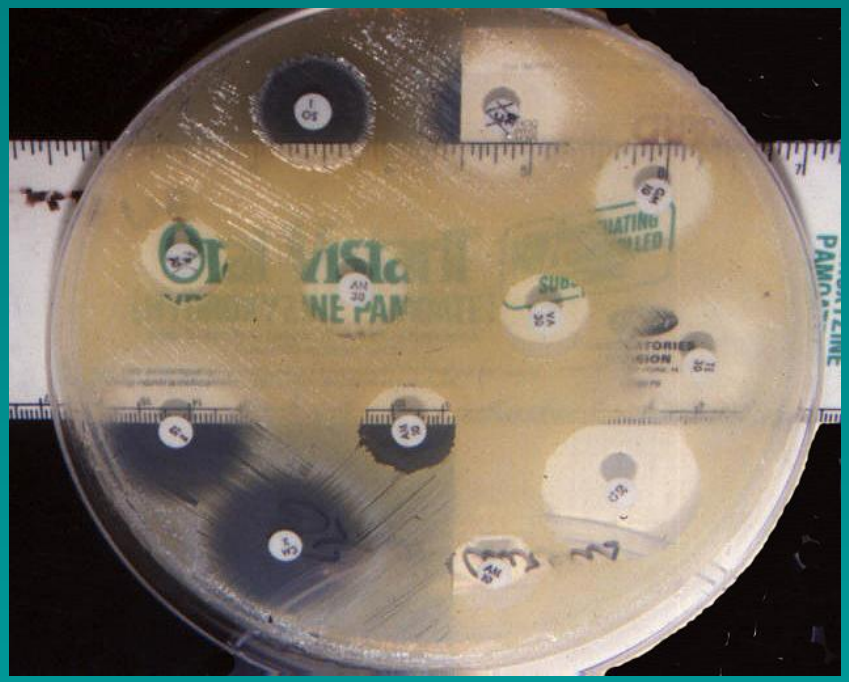
MIC = 8 ug/ml
MBC = 16 ug/ml

Susceptibility Tests

(cont'd)

3. Agar diffusion

✔ Kirby-Bauer Disk Diffusion Test



Susceptibility Tests

"Kirby-Bauer Disk-plate test" (cont'd)

Diffusion depends upon:

1. Concentration
2. Molecular weight
3. Water solubility
4. pH and ionization
5. Binding to agar

Susceptibility Tests

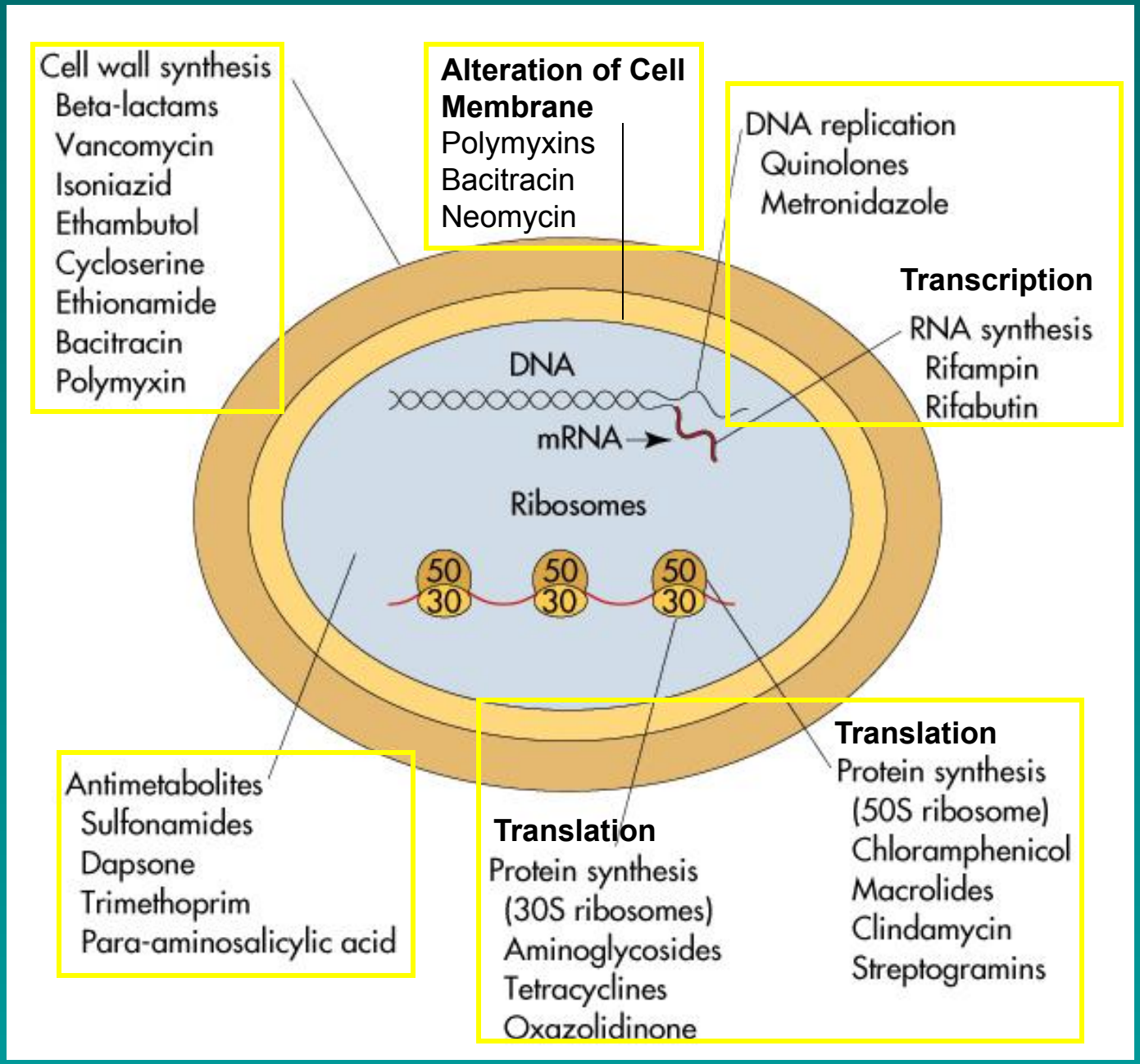
"Kirby-Bauer Disk-plate test"

(cont'd)

Zones of Inhibition (~ antimicrobial activity) depend upon:

1. pH of environment
2. Media components
   - ✔ Agar depth, nutrients
3. Stability of drug
4. Size of inoculum
5. Length of incubation
6. Metabolic activity of organisms

Antibiotic Mechanisms of Action



Mechanism of Action

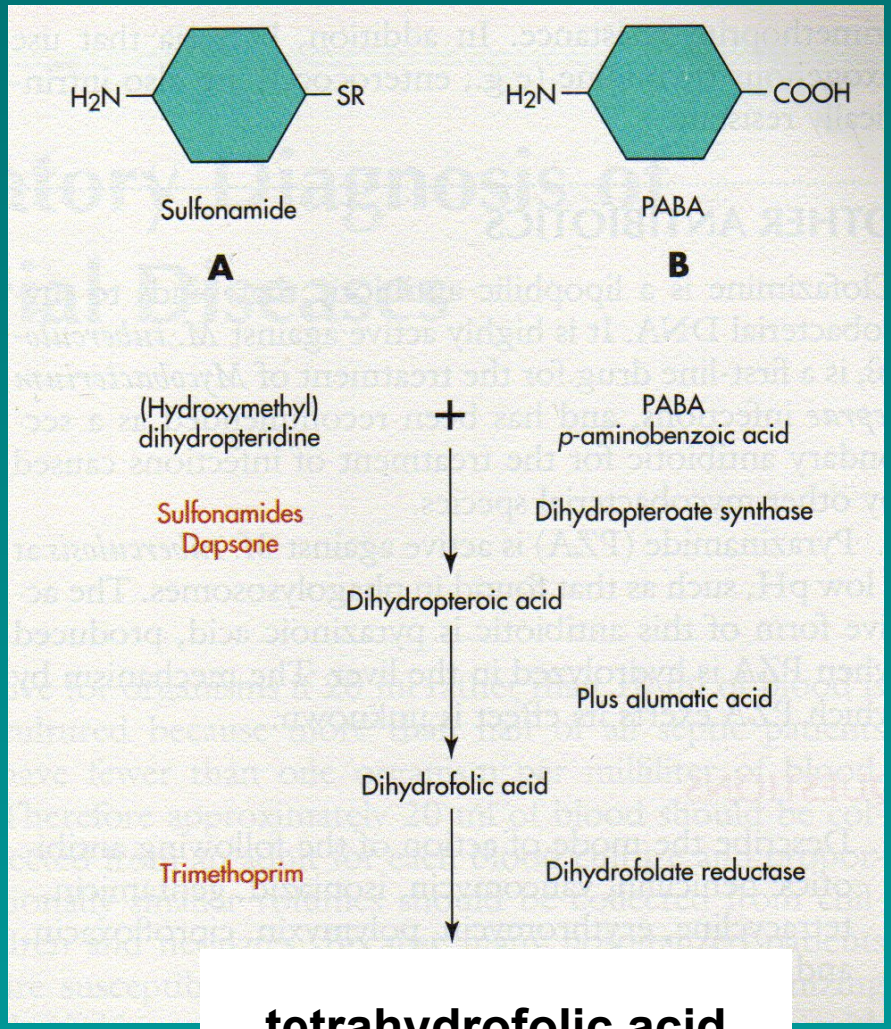
1. ANTIMETABOLITE ACTION

- **Sulfonamides**
  - ✔ an analog of PABA, works by competitive inhibition

- **Trimethoprim-sulfamethoxazole**
  - ✔ a synergistic combination; useful against UTIs

Mechanism of Action

ANTIMETABOLITE ACTION (cont'd)



Mechanism of Action

(cont'd)

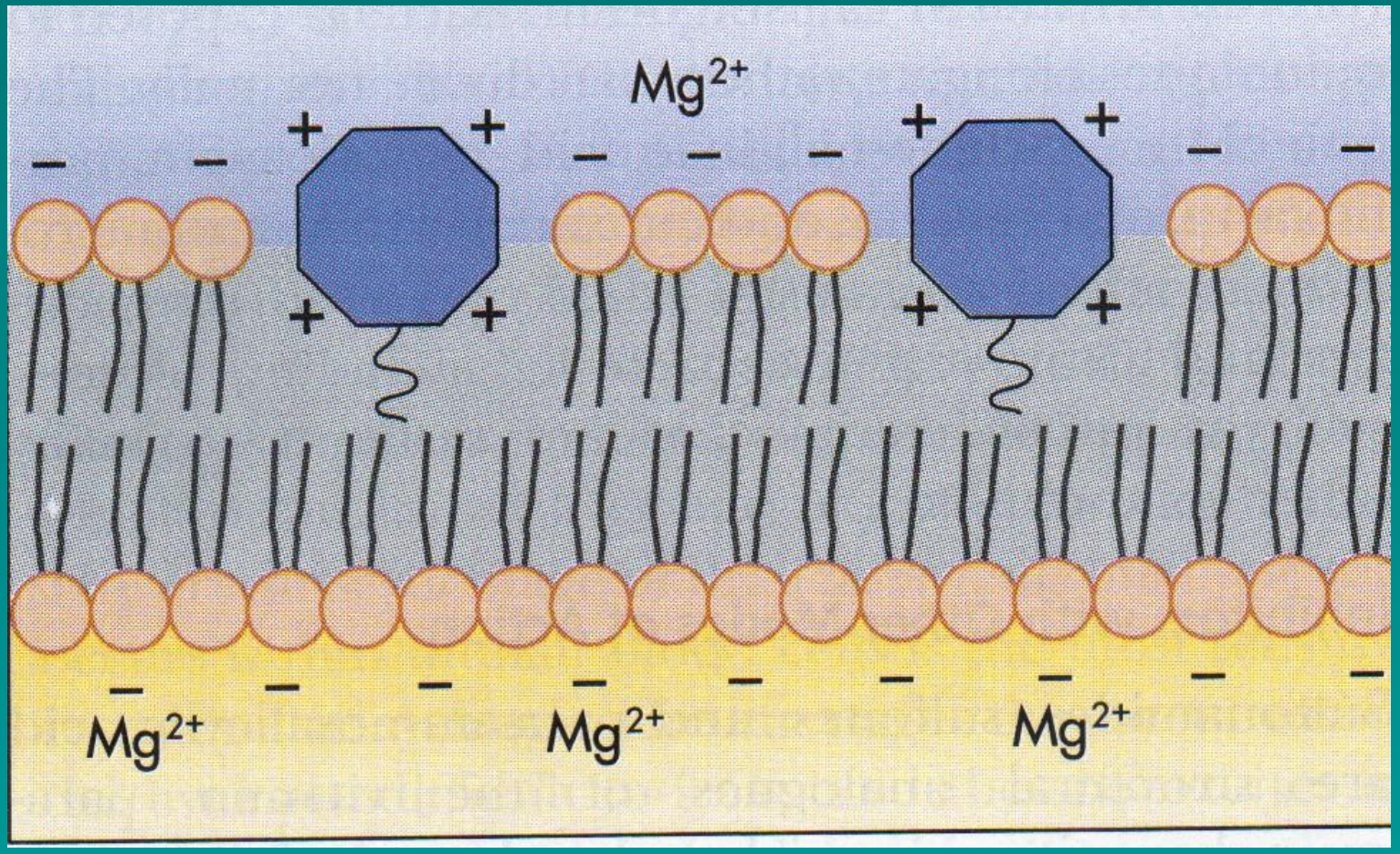
2.ALTERATION OF CELL MEMBRANES

- **Polymyxins and colistin**
  - ✔ destroys membranes
  - ✔ active against gram negative bacilli
  - ✔ serious side effects
  - ✔ used mostly for skin & eye infections

Mechanism of Action

ALTERATION OF CELL MEMBRANES

(cont'd)



Mechanism of Action

(cont'd)

3. INHIBITION OF PROTEIN SYNTHESIS:

Steps in synthesis:

1. Initiation
2. Elongation
3. Translocation
4. Termination

- Prokaryotes and eukaryotes (80S) have a different structure to ribosomes so can use antibiotics for selective toxicity against ribosomes of prokaryotes (70S)

Mechanism of Action

INHIBITION OF PROTEIN SYNTHESIS (cont'd)

- **Aminoglycosides**
  - ✔ bind to bacterial ribosome on 30S subunit; and blocks formation of initiation complex. Both actions lead to mis-incorporation of amino acids
  - ✔ Examples:

Gentamicin	Tobramycin
Amikacin	Streptomycin
Kanamycin	Spectinomycin
Neomycin	

Mechanism of Action

INHIBITION OF PROTEIN SYNTHESIS

(cont'd)

- **Aminoglycosides** (cont'd)
  - ✔ broad spectrum
    - Gram negative rods
    - *P. aeruginosa*
    - Drug-resistant gram negative rods
    - Plague, Tularemia, Gonorrhea
    - Pre-op (bowel)
    - External (skin)
  - ✔ toxic at some level to eighth cranial nerve

Mechanism of Action

INHIBITION OF PROTEIN SYNTHESIS

(cont'd)

- **Macrolides: chloramphenicol & erythromycin**

✔ bind to 50S subunit and blocks the translocation step

✔ Chloramphenicol: broad spectrum

Anaerobes

Typhoid

Meningitis

✔ Erythromycin:

Mycoplasma

Legionella

S. pyogenes

Mechanism of Action

INHIBITION OF PROTEIN SYNTHESIS

(cont'd)

- **Clindamycin**
  - ✔ binds to 50S subunit and interferes with binding of the amino acid – acyl-tRNA complex and so inhibits peptidyl transferase
  - ✔ works best against
    - Staphylococcus
    - *Bacteroides* & anaerobic gram neg rods
  - ✔ Penicillin allergic people

Mechanism of Action

INHIBITION OF PROTEIN SYNTHESIS

(cont'd)

- **Tetracyclines**
  - ✔ bind to 30S subunit and interferes with the attachment of the tRNA carrying amino acids to the ribosome
  - ✔ effective against:
    - *Chlamydia*
    - *Rickettsia*
    - *Mycoplasma*
    - *Brucella*

Mechanism of Action

(cont'd)

4.INHIBITION OF DNA/RNA SYNTHESIS

- **Rifampin**
  - ✔ binds to RNA polymerase
  - ✔ active against gram positive cocci
  - ✔ bactericidal for *Mycobacterium*
  - ✔ used for treatment and prevention of meningococcus

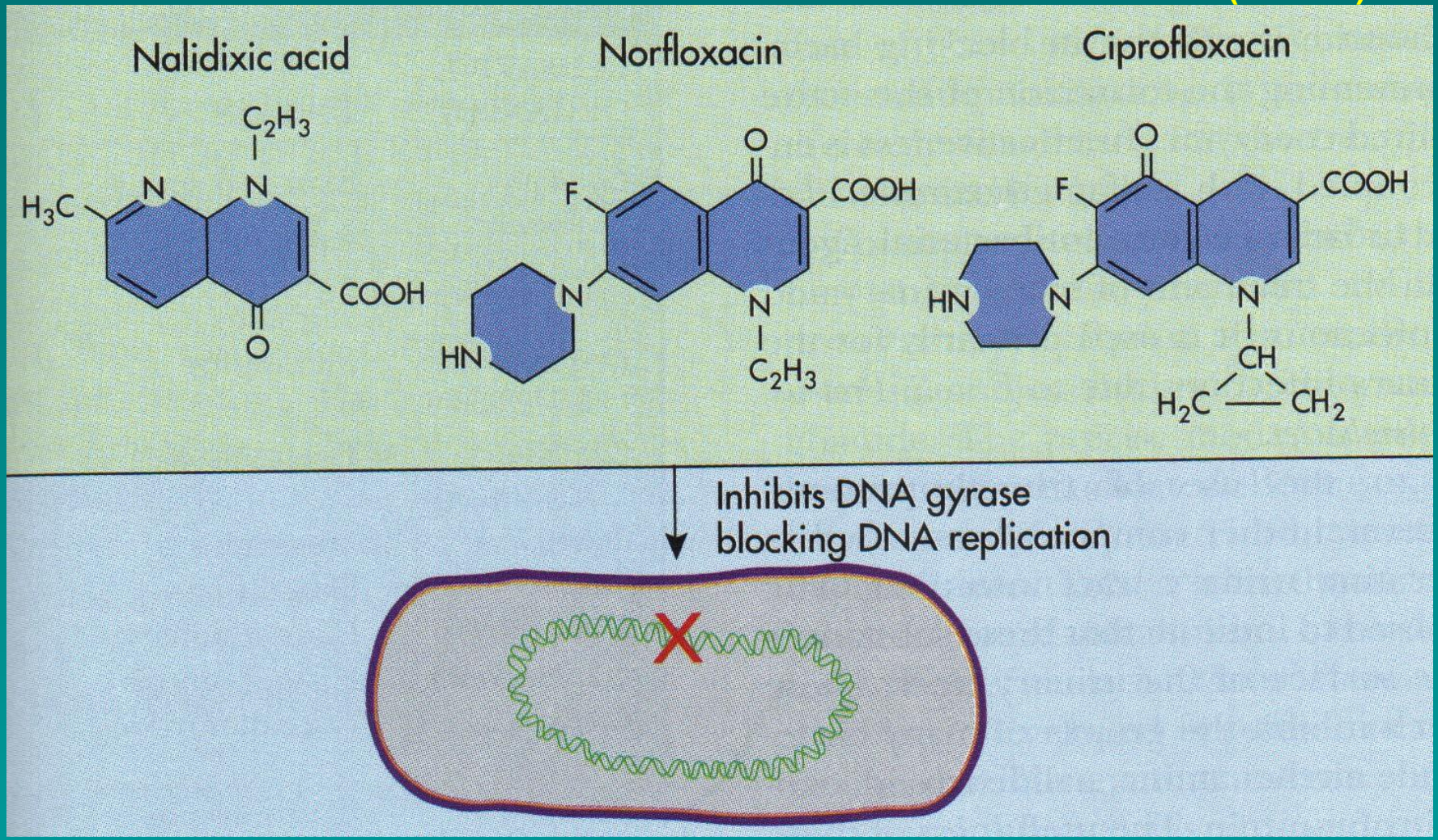
Mechanism of Action
INHIBITION OF DNA/RNA SYNTHESIS

(cont'd)

- **Metronidazole**
  - ✔ breaks down into intermediate that causes breakage of DNA
  - ✔ active against:
    - – protozoan infections
    - – anaerobic gram negative infections
- **Quinolones and fluoroquinolones**
  - ✔ effect DNA gyrase
  - ✔ broad spectrum

Mechanism of Action

INHIBITION OF DNA/RNA SYNTHESIS (cont'd)



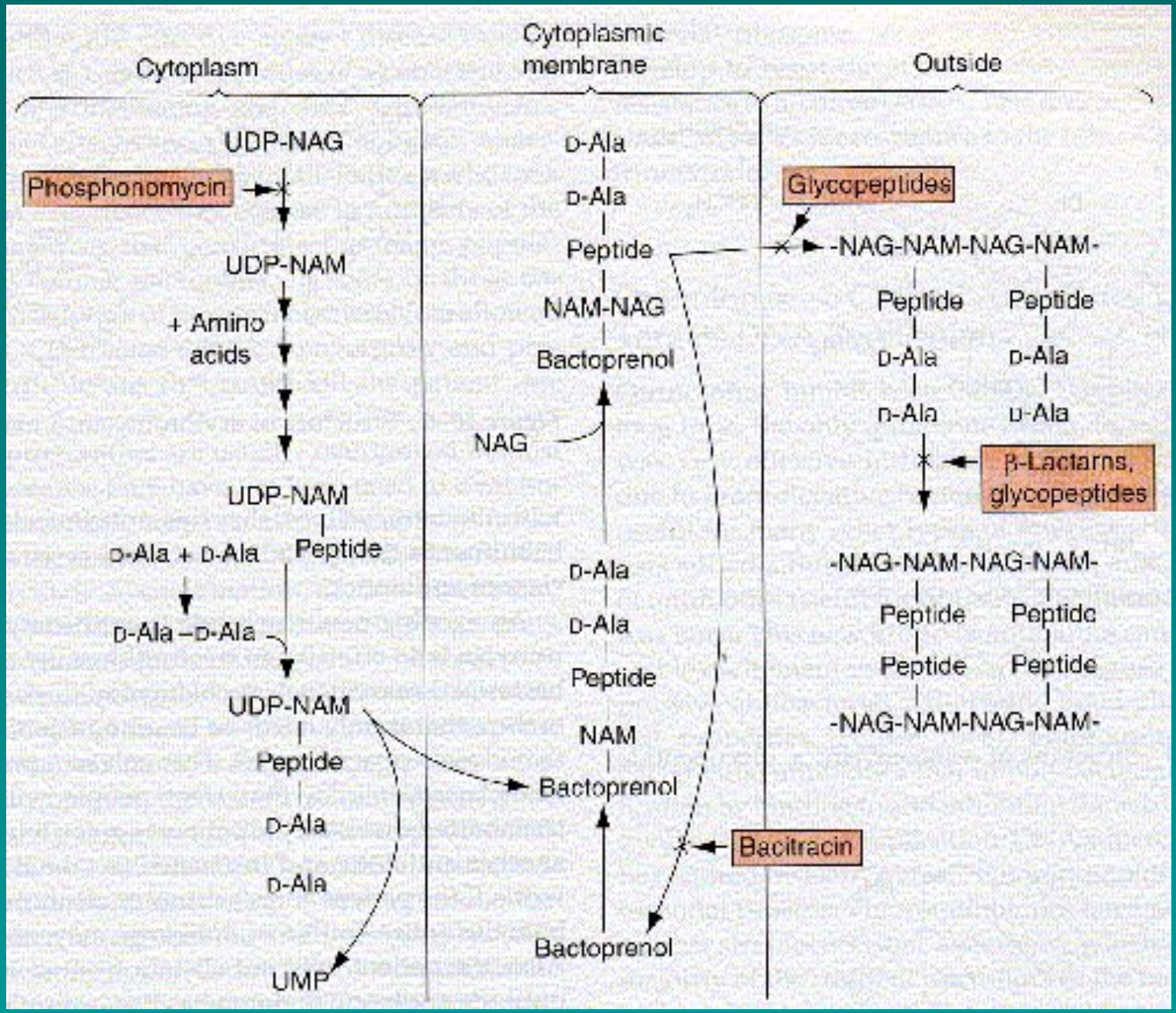
Mechanism of Action (cont'd)

5. CELL WALL SYNTHESIS INHIBITORS

   Steps in synthesis:

   1. NAM-peptide made in cytoplasm
   2. attached to bactoprenol in cell membrane
   3. NAG is added
   4. whole piece is added to growing cell wall
   5. crosslinks added

- the β-Lactams
- the non β-Lactams


Cytoplasm
Cytoplasmic membrane
Outside
UDP-NAG
Phosphonomycin
UDP-NAM
+ Amino acids
UDP-NAM
Peptide
D-Ala + D-Ala
D-Ala–D-Ala
UDP-NAM
Peptide
D-Ala
D-Ala
UMP
D-Ala
D-Ala
Peptide
NAM-NAG
Bactoprenol
NAG
D-Ala
D-Ala
Peptide
NAM
Bactoprenol
Bactoprenol
Glycopeptides
-NAG-NAM-NAG-NAM-
Peptide
Peptide
D-Ala
D-Ala
D-Ala
D-Ala
β-Lactams, glycopeptides
-NAG-NAM-NAG-NAM-
Peptide
Peptide
Peptide
Peptide
-NAG-NAM-NAG-NAM-
Bacitracin

Mechanism of Action (cont'd)

5. CELL WALL SYNTHESIS INHIBITORS

   β-Lactam Antibiotics

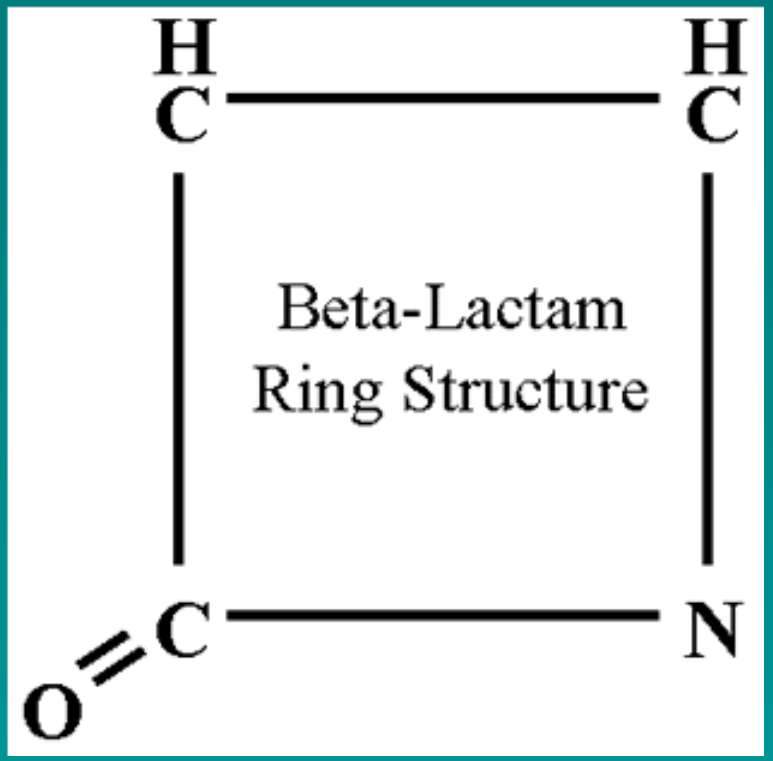
   - ✔ Penicillins
   - ✔ Cephalosporins
   - ✔ Carbapenems
   - ✔ Monobactams

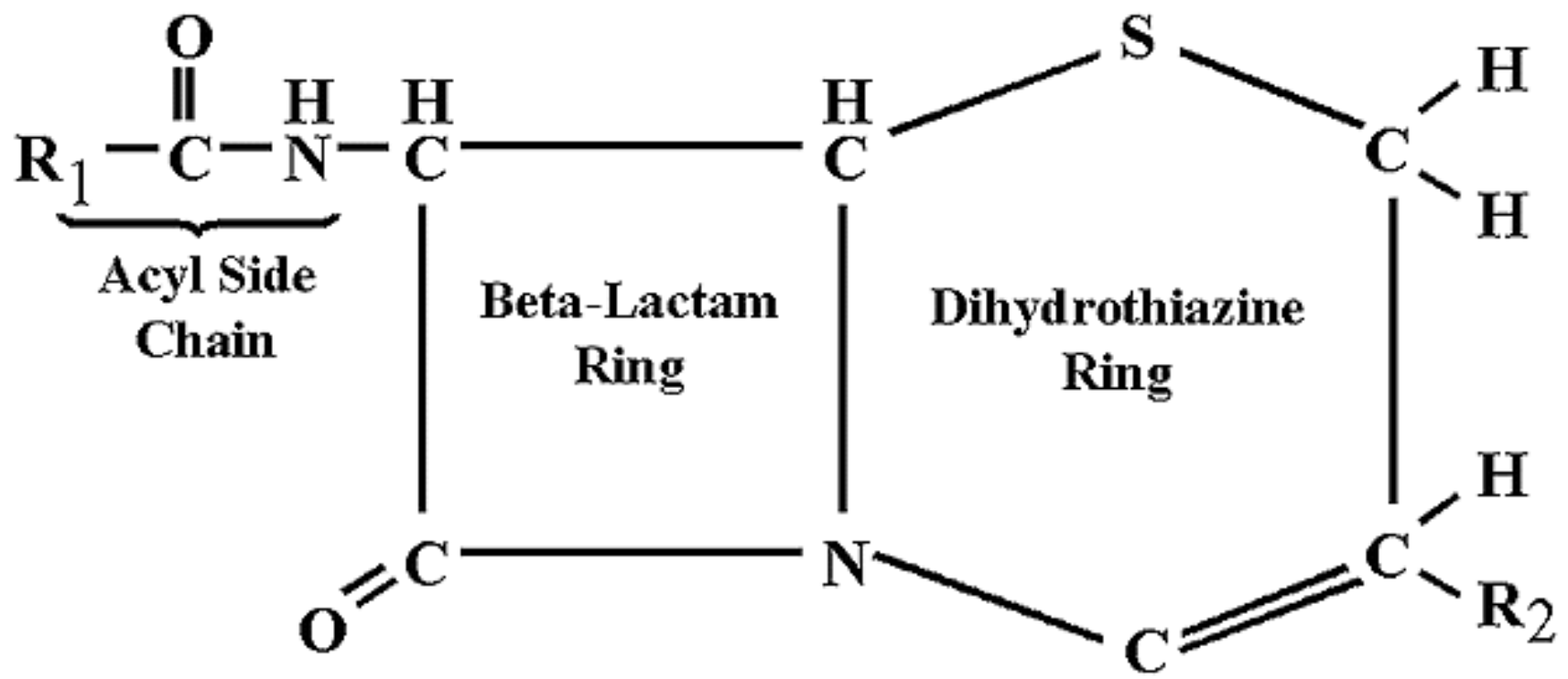
Mechanism of Action

CELL WALL SYNTHESIS INHIBITORS

(cont'd)

β-Lactam ring structure





General Structure of Cephalosporins

Mechanism of Action

CELL WALL SYNTHESIS INHIBITORS

(cont'd)

Action of β-Lactam antibiotics

1. Bactericidal; growing cells only
2. Drug links covalently to regulatory enzymes called PBPs (penicillin-binding proteins)
3. Blocks cross-linkage of peptidoglycan

Mechanism of Action

CELL WALL SYNTHESIS INHIBITORS

(cont'd)

Action of β-Lactam antibiotics

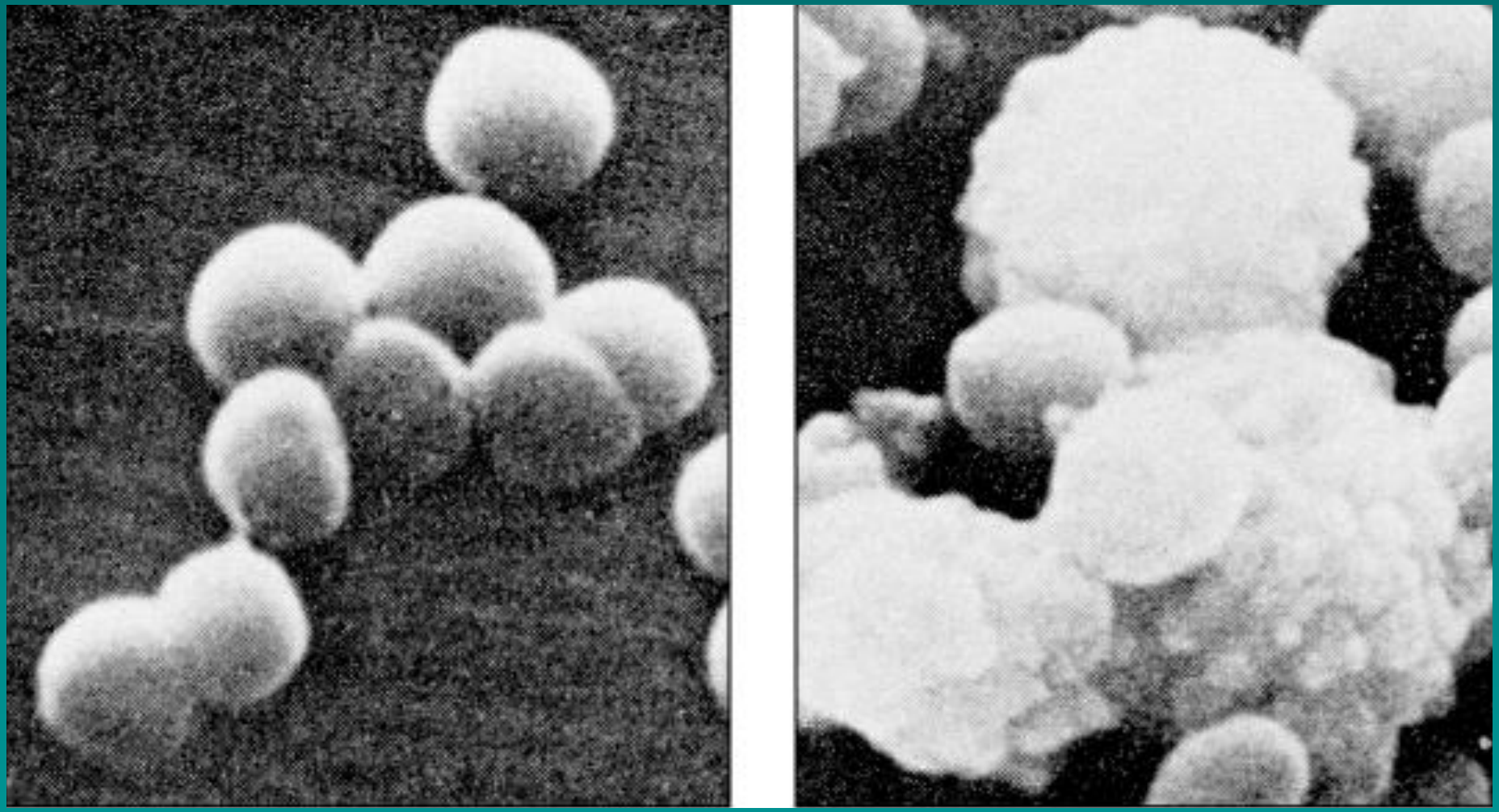
For E. coli

> MIC

- wall damage
- autolysins
- spheroplasting
- cell lysis

< MIC

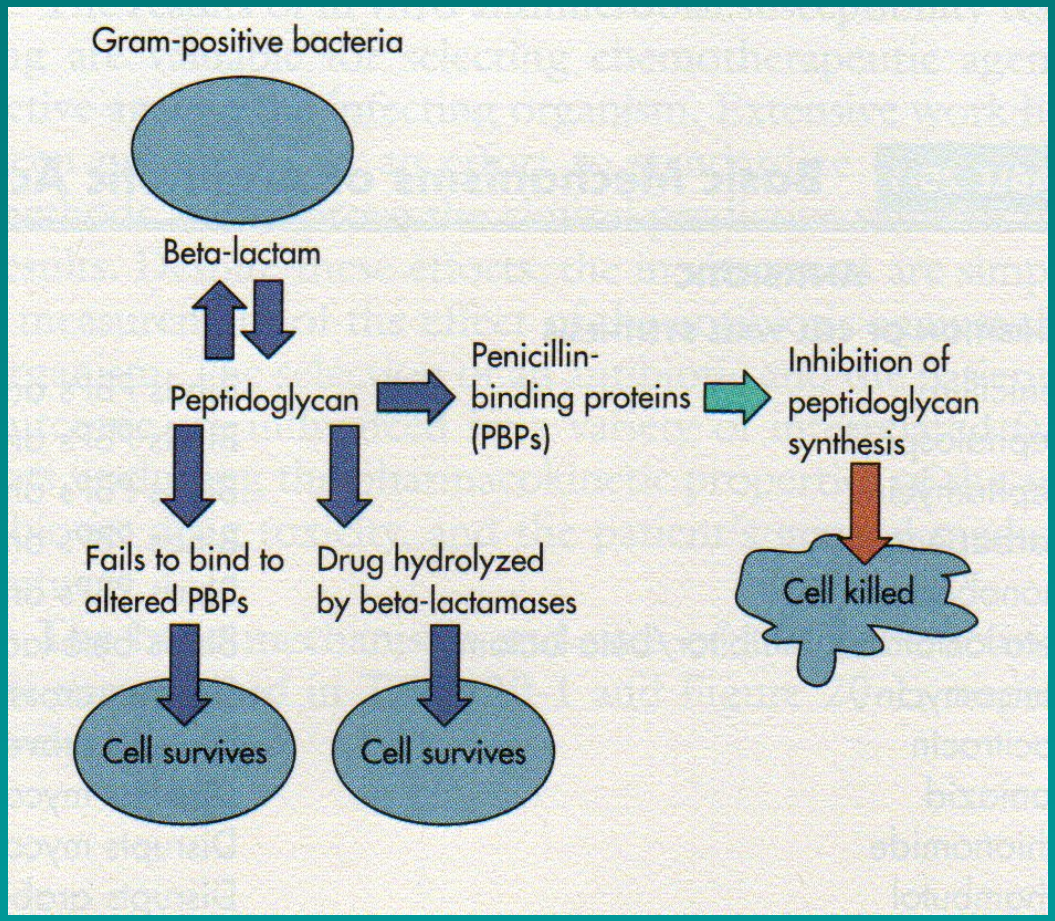
- no septa
- filaments



Mechanism of Action

CELL WALL SYNTHESIS INHIBITORS

Resistance to β-Lactams – Gram pos. (cont'd)

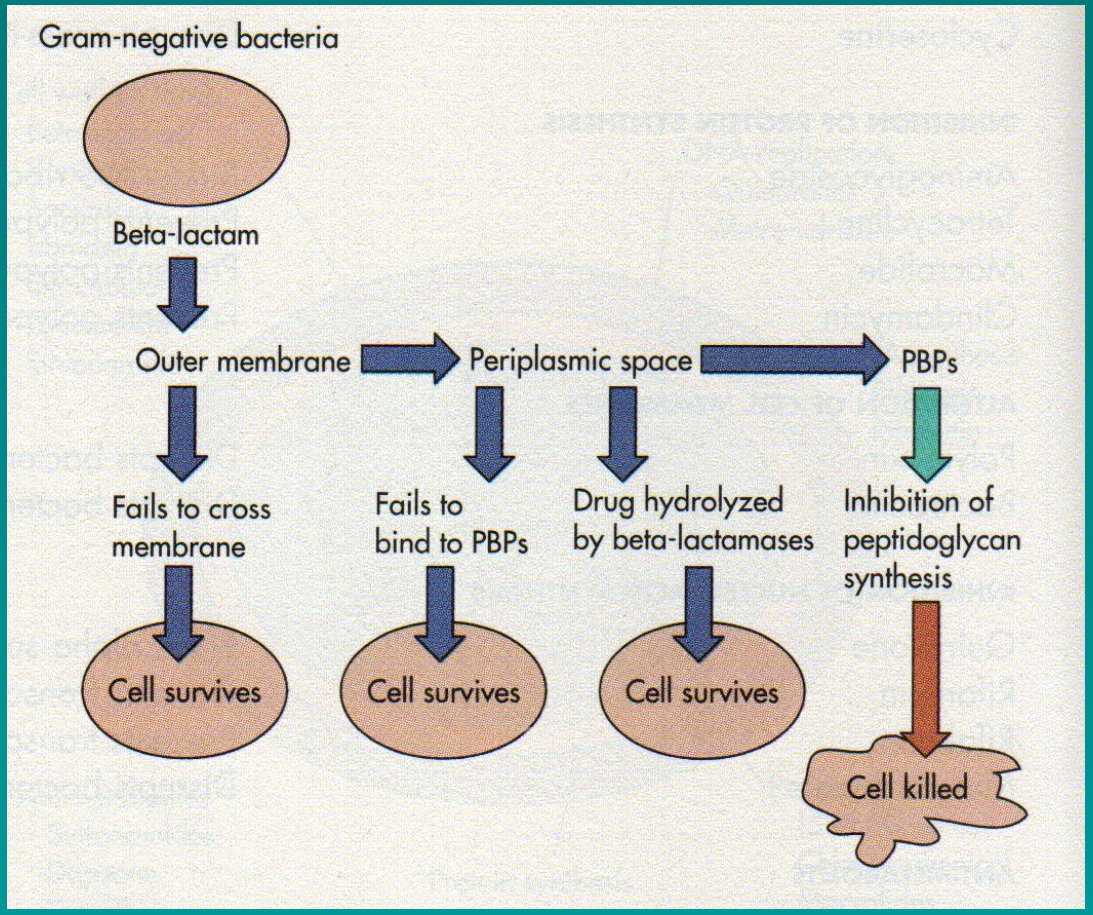


Mechanism of Action

CELL WALL SYNTHESIS INHIBITORS

(cont'd)

Resistance to β-Lactams – Gram neg.



Mechanism of Action

CELL WALL SYNTHESIS INHIBITORS

(cont'd)

Non - β-Lactams

- **Vancomycin**

✔active against gram positive cocci, but not gram negative because too large to pass through outer membrane

✔ interferes with PG elongation

- **Cycloserine, ethionamide and isoniazid**

✔inhibits enzymes that catalyze cell wall synthesis

✔for *Mycobacterial* infections

Clinical Uses

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

Clinical Uses (cont'd)

PATHOGENS	TYPICAL DRUG
Mycobacterium	Streptomycin Iso-nicotinic hydrazide (INH)
Fungi: Cutaneous Deep	 Nystatin Amphotericin B, ketoconazol
Parasites: Plasmodium Giardia	 Chloroquine Quinacrine

Resistance
Physiological Mechanisms

1. Lack of entry – tet, fosfomycin
2. Greater exit
   - ✔ efflux pumps
   - ✔ tet (R factors)
3. Enzymatic inactivation
   - ✔ bla (penase) – hydrolysis
   - ✔ CAT – chloramphenicol acetyl transferase
   - ✔ Aminogylcosides & transferases

Resistance
Physiological Mechanisms
(cont'd)

4. Altered target
   - ✔ RIF – altered RNA polymerase (mutants)
   - ✔ NAL – altered DNA gyrase
   - ✔ STR – altered ribosomal proteins
   - ✔ ERY – methylation of 23S rRNA
5. Synthesis of resistant pathway
   - ✔ TMP^r plasmid has gene for DHF reductase; insensitive to TMP

Origin of Drug Resistance

- Non-genetic
  - ✔ metabolic inactivity
    - *Mycobacteria*
  - ✔ non-genetic loss of target
    - penicillin – non-growing cells, L-forms
  - ✔ intrinsic resistance
    - some species naturally insensitive

Origin of Drug Resistance

(cont'd)

- Genetic
  - ✔ spontaneous mutation of old genes
    - Vertical evolution
  - ✔ Acquisition of new genes
    - Horizontal evolution

- Chromosomal Resistance
- Extrachromosomal Resistance
  - Plasmids, Transposons, Integrons

Plasmids

- independent replicons
  - ✔ circular DNA
- dispensable
- several genes
  - ✔ drug resistance
  - ✔ metabolic enzymes
  - ✔ virulence factors
- host range
  - ✔ restricted or broad

Plasmids (cont'd)

- size
  - ✔ small, non-conjugal
  - ✔ large, conjugal <25 kbp
- Transfer between cells:
  - ✔ CONJUGATION (cell to cell contact)
    - due to plasmid tra genes (for pili, etc)
  - ✔ NON-CONJUGAL
    - transduction
    - mobilization by conjugation plasmids

Implications of Resistance

- Household agents
  - ✔ they inhibit bacterial growth
  - ✔ purpose is to prevent transmission of disease-causing microbes to noninfected persons.
  - ✔ can select for resistant strains
- NO evidence that they are useful in a healthy household

Implications of Resistance

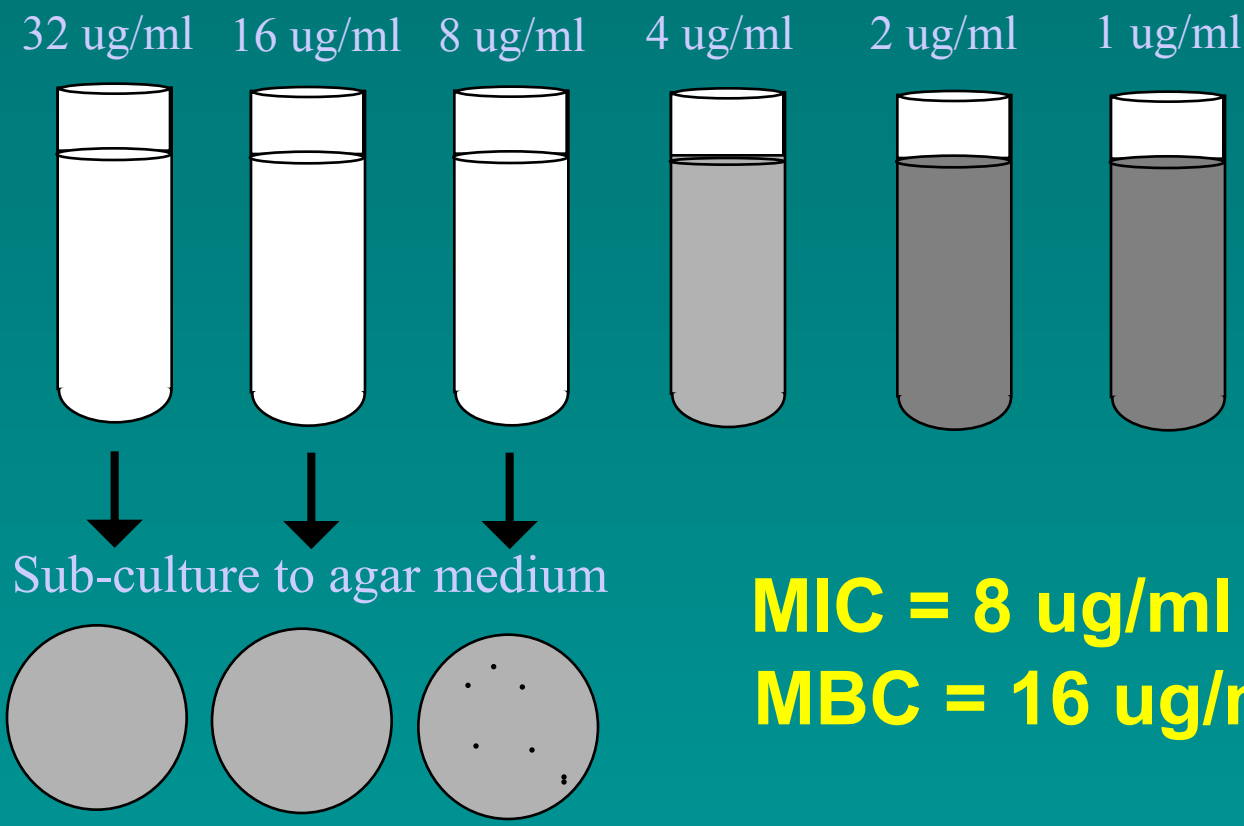
- Triclosan studies
  - ✔ effect diluted by water
  - ✔ one gene mutation for resistance
  - ✔ contact time exceeds normal handwash time (5 seconds)
- Allergies
  - ✔ link between too much hygiene and increased allergy frequency
- http://www.healthsci.tufts.edu/apua/ROAR/roarhome.htm

Implications of Resistance

- www.roar.apua.org

REVIEW

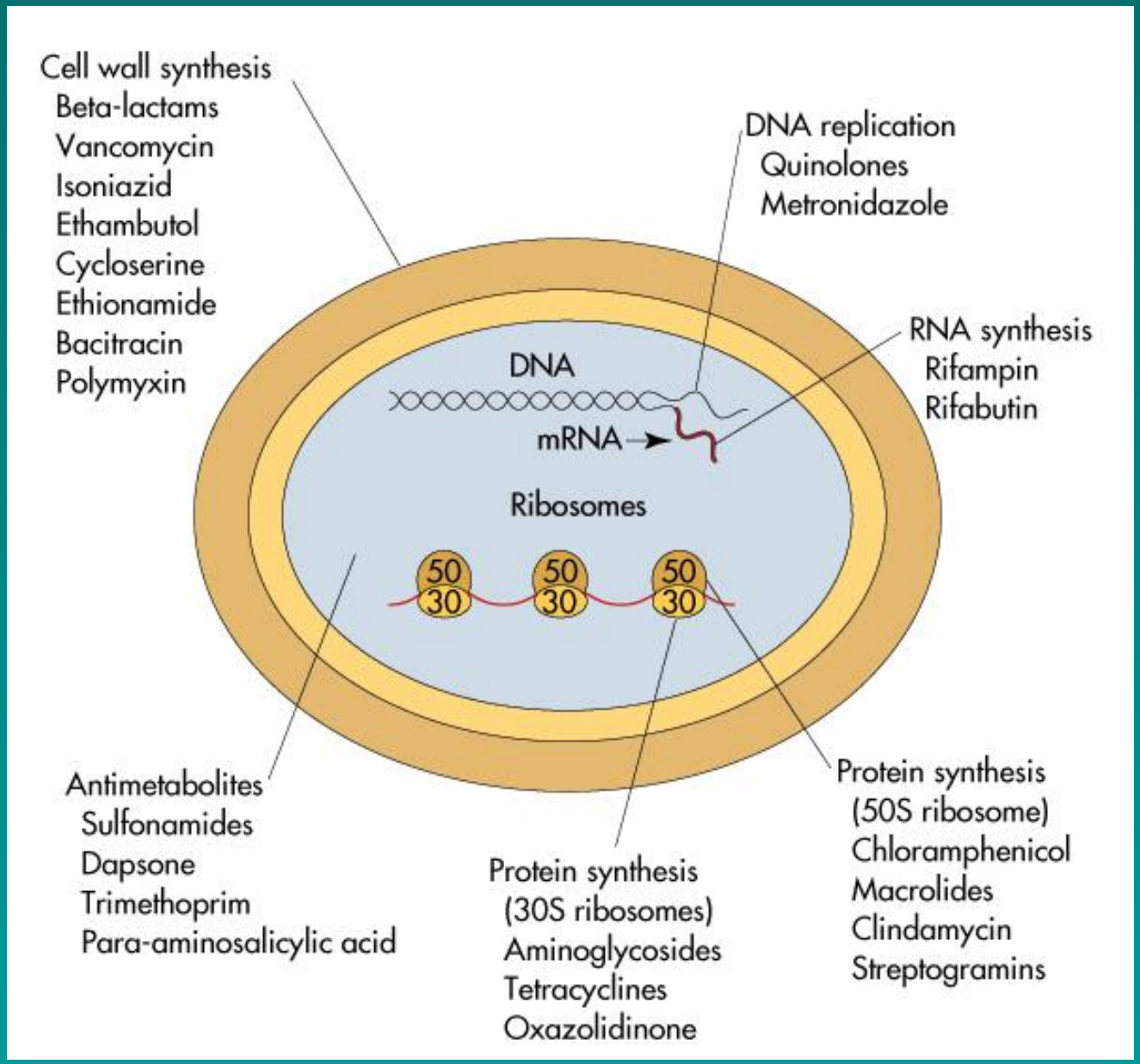
Minimal Inhibitory Concentration (MIC)
vs.
Minimal Bactericidal Concentration (MBC)



MIC = 8 ug/ml
MBC = 16 ug/ml

REVIEW

What are main targets of Antibiotics?



REVIE
W

Mechanism of Action

INHIBITION OF CELL WALL SYNTHESIS

- **β-Lactams**
- **Non β-Lactams**

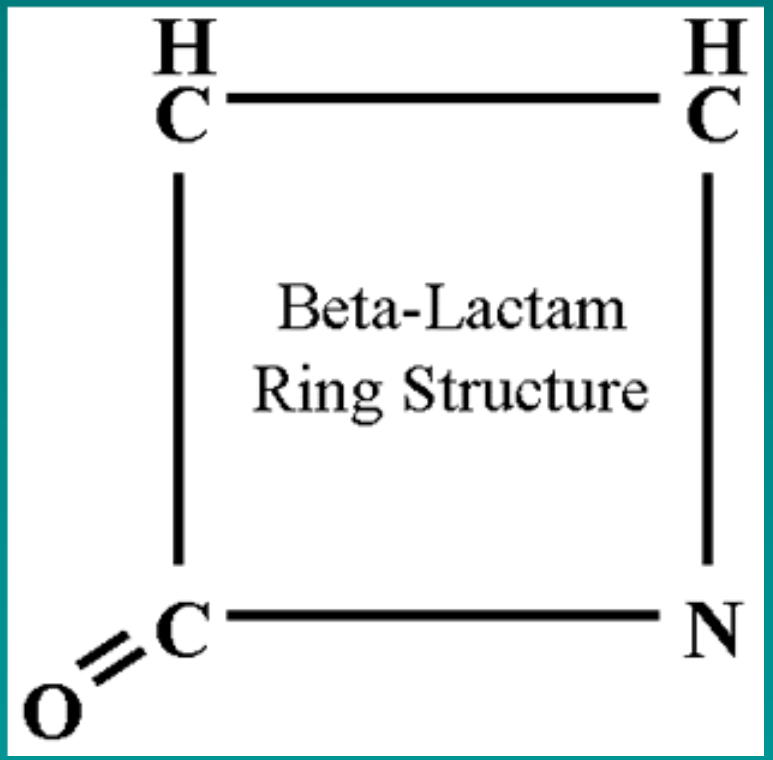
REVIEW

Mechanism of Action

CELL WALL SYNTHESIS INHIBITORS

(cont'd)

β-Lactam ring structure



REVIEW

Mechanism of Action

INHIBITION OF PROTEIN SYNTHESIS

- Aminoglycosides
- Macrolides
  - ✔ Chloramphenicol
  - ✔ Erythromycin
- Tetracyclines
- Clindamycin

REVIEW

Mechanism of Action

INHIBITION OF NUCLEIC ACID SYNTHESIS

- Rifampin
- Metronidazole
- Quinolones and fluoroquinolones

REVIEW

Mechanism of Action

DISRUPTION OF CELL MEMBRANES

- **Polymyxins**
- **Colistin**

REVIEW

Mechanism of Action

ANTIMETABOLITE ACTION

- **Sulfonamides**
- **Trimethoprim-sulfamethoxazole**

REVIEW

Resistance
Physiological Mechanisms

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REVIEW

Resistance
Physiological Mechanisms

(cont'd)

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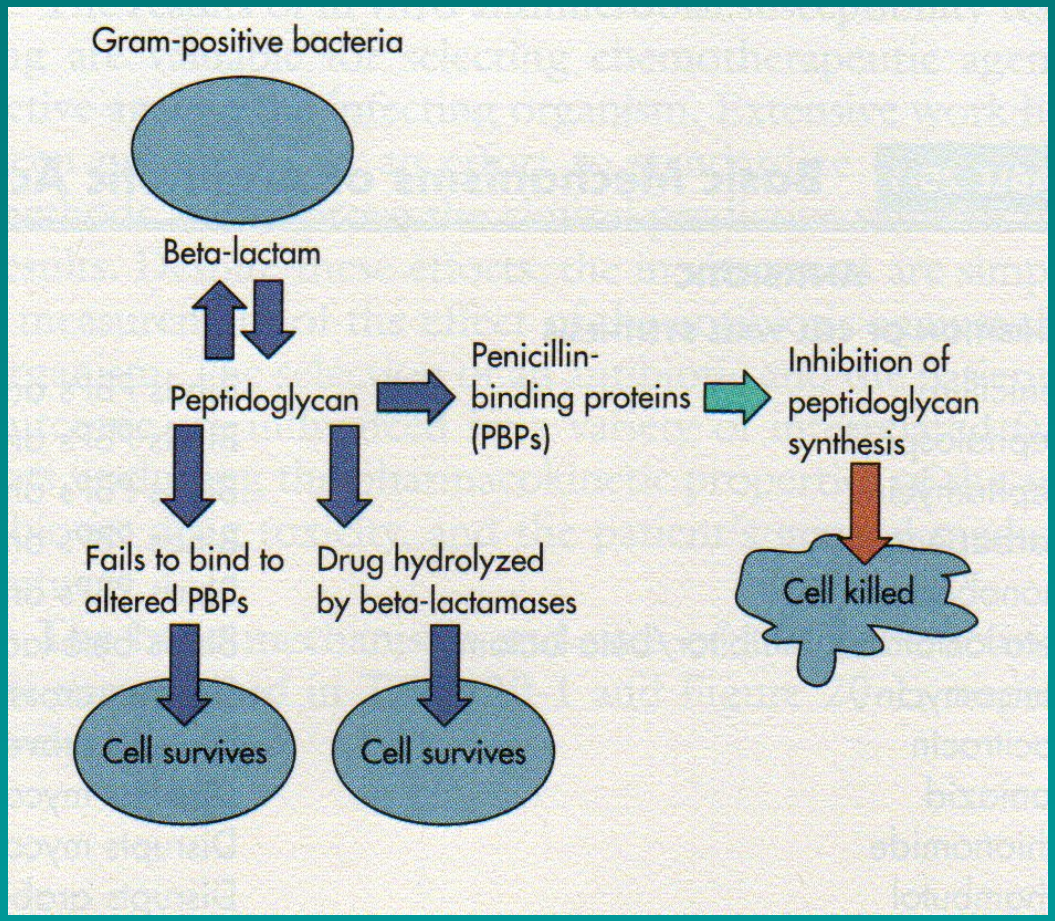
REVIEW

Mechanism of Action

CELL WALL SYNTHESIS INHIBITORS

Resistance to β-Lactams – Gram pos. (cont'd)



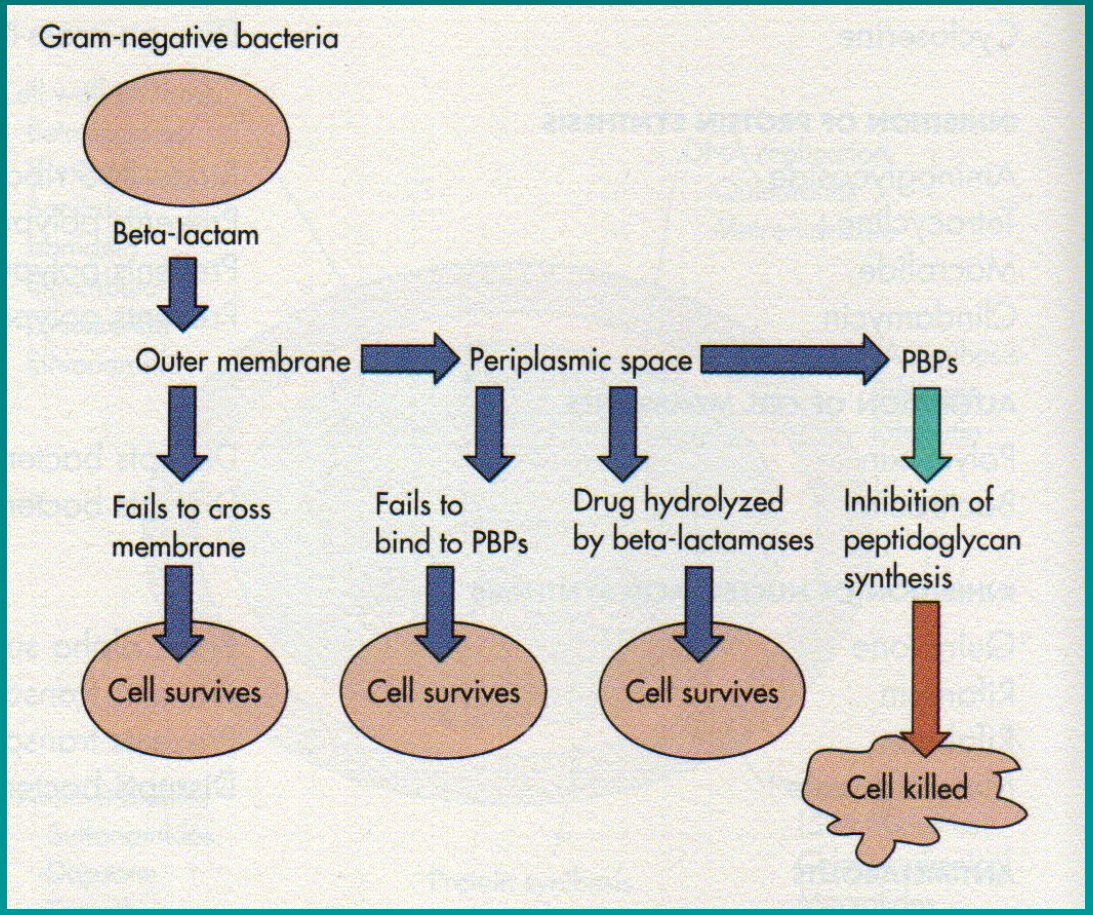
REVIEW

Mechanism of Action

CELL WALL SYNTHESIS INHIBITORS

(cont'd)

Resistance to β-Lactams – Gram neg.



REVIEW