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جامعة الملك سعود
كلية العلوم
قسم الكيمياء الحيوية

المضادات الحيوية (BCH 476)

Antibiotics

Lecture 14-16: Mechanism of action of antibiotics

Inhibitors of cell wall synthesis

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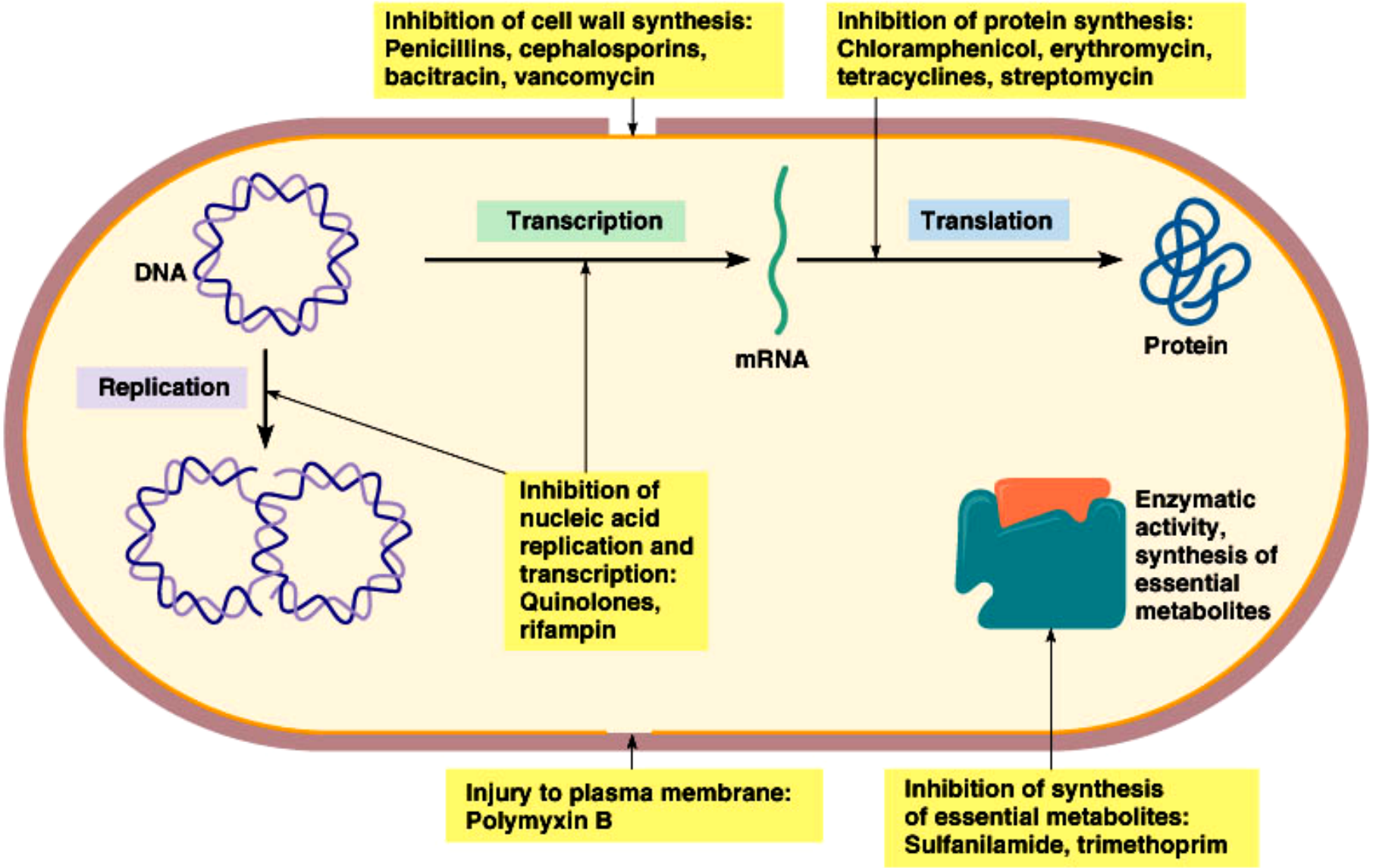
Lecture No.	Topics
14-16	**Mechanism of action of antibiotics:** -Biochemical targets or sites of action of antibiotics -Inhibitors of cell wall synthesis (e.g. penicillin, cephalosporin, cycloserine, phosphonomycin).

Mechanism of action of antibiotics

Biochemical targets or sites of action of antibiotics:

- Inhibitors of cell wall synthesis
- Antibiotics affecting membrane structure & function
- Antibiotics affecting purine & pyrimidine synthesis
- Antibiotics inhibiting nucleic acid synthesis
    - Inhibitors of RNA metabolism
    - Inhibitors of DNA metabolism
- Protein biosynthesis-inhibition by antibiotics
- Energy metabolism-inhibiting antibiotic (Oxidative phosphorylation & respiratory chain inhibitors)
- Antibiotics as antimetabolites



Reasons of studying mechanism of action

Scientists study antibiotics to answer the following question? (Gale 1966):

-What are the precise mechanisms of the toxic action?

-What is the site of the toxic action within the sensitive cell?

-Why is the action selective?

-What is the relationship between the chemical structure of the drug and the chemistry of the sensitive site?

-By what mechanisms do normally sensitive cells become resistant to toxic action?

Biochemical targets for antibiotic action

- Antibiotics that affect growth, metabolism and multiplication of certain type of cells may interact with various targets inside the sensitive cells.
- **Is it better to take high concentration of antibiotic or lower concentration?**
- An efficient antibiotic must be given in a concentration that affect few targets or only one target, to be safe for normal cells.
- By lowering antibiotic concentration, it will be harmful to only one target and safe for others.

Antibiotics and the selective toxicity

- An antibiotic to be marketed it must exhibit selective toxicity.
- This means that the drug is harmful to the microbe and safe to the host. The term selective toxicity is relative rather than absolute; this means that a drug in a concentration tolerated by host may damage an infecting microorganism.
- The selective toxicity may be due to the existence of specific antibiotic receptor in the microbe that is not present in the host cells.

As metabolic process, growth and multiplication depend on enzymatic reaction, a number of possible antibiotic mode of action can exist:

- Direct action at the substrate binding site by competitive inhibition (when the antibiotic structure is similar, analogue, to the substrate structure) or by false feed back inhibition when it is similar to the product or it may attack some element in the enzyme active site like –SH group.
- Direct action at the cofactor binding site
- Modification of substrate binding by false operation of the specific systems that regulate enzyme-substrate interaction
- Disorganization of the active center by non-specific action elsewhere in the enzyme.

Antibiotics that inhibit cell wall synthesis

- Animal cells do not contain cell wall, instead it has cell membrane.
- Bacteria, fungi and plant contain rigid cell wall:
  - Bacteria contain peptidoglycan
  - Fungi contain chitin
  - Plants contain cellulose.
- Several antibiotic actions depend on its ability to inhibit the biosynthesis of cell wall.

Composition of bacterial cell wall

- Several prokaryotes contain some characteristic polymers such as:
  - peptidoglycan,
  - teicholic acid,
  - teichuronic acid and
  - lipopolysaccharides.
- The most abundant polymer in gram positive bacteria is peptidoglycan which is heteropolymer containing several types of subunits.
- Each microorganism has its characteristic peptidoglycan structure.

- Bacteria possess a rigid outer layer, the cell wall. It maintain the shape of the microorganism and protect it from explosion because the internal pressure of G+ve bacteria is five times greater than the pressure in G–ve ones.
- This cell wall protect bacteria from the access of some drugs and the acidic pH of the stomach.
- In a hypertonic solution (high osmolarity) like 20% sucrose, the cell wall will be damaged leading to the formation of spherical G +ve bacteria "protoplast" or G-ve bacteria "spheroplast".
- These naked bacteria take up fluid rapidly and swell and may explode.

- In both Gram-positive and Gram-negative bacteria the cell wall is formed from a cross-linked chain of alternating units of:
  - N-acetylglucosamine and
  - N-acetylmuramic acid,
- They are known as peptidoglycan or mucopeptide.
- In Gram-positive organisms the cell wall structure is thick (about 30 nm), tightly cross-linked, and interspersed with polysugar phosphates (teichoic acids), some of which have a lipophilic tail buried in the cell membrane (lipoteichoic acids).
- Gram-negative bacteria, in contrast, have a relatively thin (2-3 nm), loosely cross-linked peptidoglycan layer and have no teichoic acid.

Compare between the structure of cell wall of Gram positive and Gram negative bacteria

- Home work

Synthesis of bacterial cell wall

1- N-acetylmuramic acid is manufactured from N-acetylglucosamine by the addition of lactic acid derived from phosphoenolpyruvate.

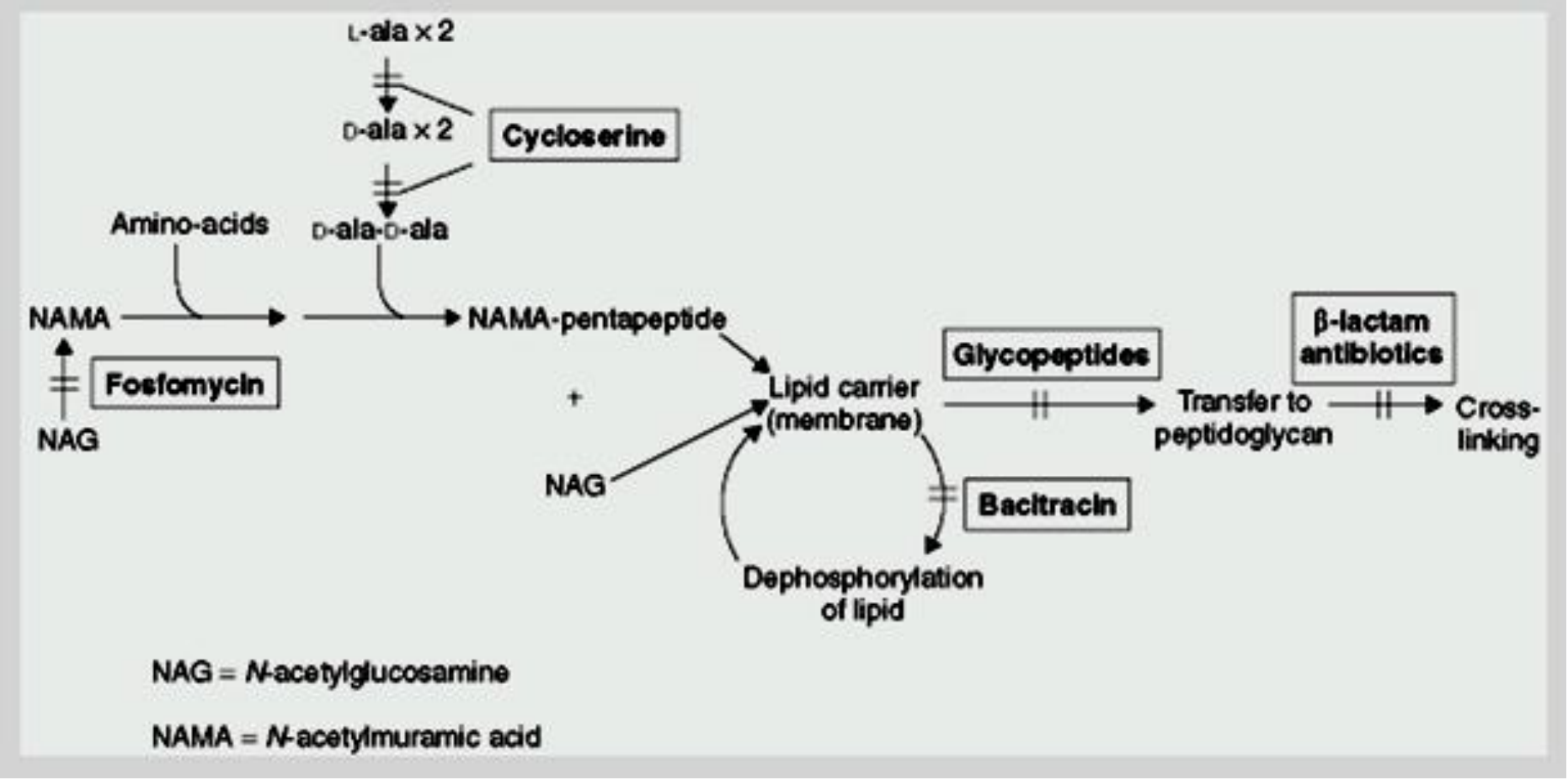
2- Three amino acids are then added to N-acetylmuramic acid to form a muramic acid tripeptide.

3- Meanwhile, two D-alanine residues, produced from L-alanine by an enzyme called alanine racemase, are joined together by another enzyme, D-alanine synthetase. These D-ala-D-ala, is added to the tripeptide to form pentapeptide of muramic acid.

4- The pentapeptide of muramic acid is joined to other N-acetylglucosamine molecule and passed to a lipid carrier in the cell membrane.

5- The whole building block of the newly synthesized soluble peptidoglycan is transported across the cell membrane and added to the end of the existing cell wall insoluble crosslinked peptidoglycan.

6- Finally, adjacent units are cross-linked to give more strong crosslinked insoluble peptidoglycan (the cell wall).



Summary of peptidoglyan synthesis and the antibiotics affecting this process.

Antibiotics affecting cell wall synthesis

- Several groups of antibiotics, notably β-lactam agents (penicillins, cephalosporins, and their relatives) and glycopeptides (vancomycin and teicoplanin) affect peptidoglycan cell wall synthesis.

Site of action of bacterial-cell wall inhibiting antibiotics

Bacterial peptidoglycans are synthesized by three stages:

- **Stage 1** occurs in the cytoplasm, the backbone structure of murein (N-acetylglucosamine and N-acetyl-muramyl pentapeptide) are synthesized with the addition of pentapeptides.
- The antibiotic called **fosfomycin** inhibits the formation of N-acetylmuramic acid from N-acetylglucosamine. Also the antibiotic, **D-cycloserine** (a structural analog of D-alanine) inhibits alanine racemase and D-alanine synthetase by binding to the substrate binding site of the two enzymes, so it is extremely effective in preventing D-alanine from being incorporated into the N-acetyl muramic acid tripeptide.

Stage 2 occurs on the inner surface of the cytoplasmic membrane

- N-acetylmuramic pentapeptide is transferred from UDP to a carrier lipid and is then modified to form a complete nascent peptidoglycan subunit.
- The glycopeptides antibiotics, vancomycin and teicoplanin are complex heterocyclic molecules that can bind to acyl-D-alanyl-D-alanine in peptidoglycan, thereby preventing the addition of new building blocks to the growing cell wall.
- They are too bulky to penetrate the external membrane of Gram-negative bacteria, so the spectrum of activity is virtually restricted to Gram-positive organisms.
- Ristocetin acts by binding to the D-alanyl-D-alanine peptide termini of the nascent peptidoglycan-lipid carrier.
- This inhibits the enzyme transglycosylase.

- **Stage 3 includes transpeptidation, removal of D-alanine and the binding of newly synthesized soluble peptidoglycan to the preexisting, crosslinked, insoluble cell wall peptidoglycan.**

In the bacteria cell wall there are as many as seven enzymes (depending on the bacterial species) which bind peptidoglycan units via their D-alanyl-D-alanine residues.

These enzymes are called "**P**enicillin **B**inding **P**roteins" as its enzymes active site bind the D-alanyl-D-alanine end of the peptidoglycan strand or alternatively bind penicillin.

•How Fosfomycin and Cycloserine antibiotics inhibit cell wall synthesis?

Fosfomycin: is a naturally occurring antibiotic originally obtained from a species of Streptomyces.

It inhibits the pyruvyl transferase enzyme that brings about the condensation of phosphoenolpyruvate and N-acetylglucos-amine in the formation of N-acetylmuramic acid.

Cycloserine: has broad-spectrum, antibacterial activity. It is used against multiresistant Mycobacterium tuberculosis. The drug bears a structural resemblance to the D-isomer of alanine and inhibits alanine racemase. It also blocks the synthetase enzyme that links two D-ala molecules together before they are inserted into the cell wall.

- **How penicillin, cephalosporin and other β–lactam antibiotics inhibit cell wall synthesis?**

β-lactam antibiotics (like penicillin) are structural analogs of D-Ala-D-Ala termini of the immature peptidoglycan.

The cell wall enzyme binding sites can bind β-lactam ring of the antibiotic as the natural substrate (D-Ala-D-Ala-).

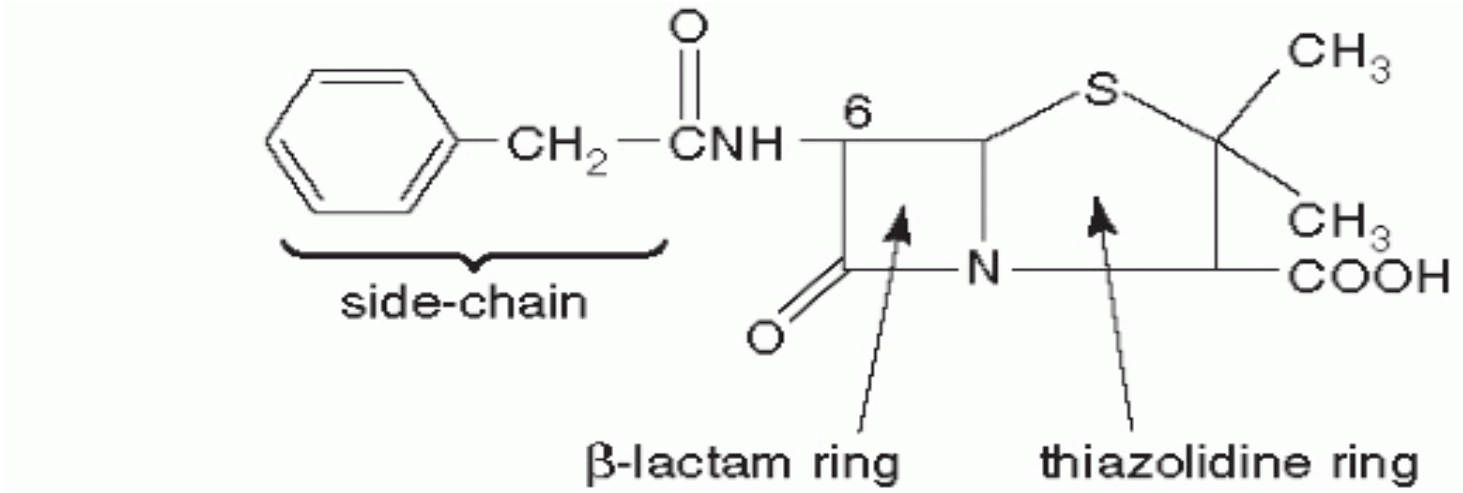
These enzymes are called Penicillin Binding Proteins (PBPs).

The β-lactams (Like Penicillin) **IRRIVERSIBLY** bind the PBPs in the enzyme active site and thus prevent the access of D-alanyl-D-alanine residues to the enzyme and so prevent transpeptidation and peptidoglycan maturation.

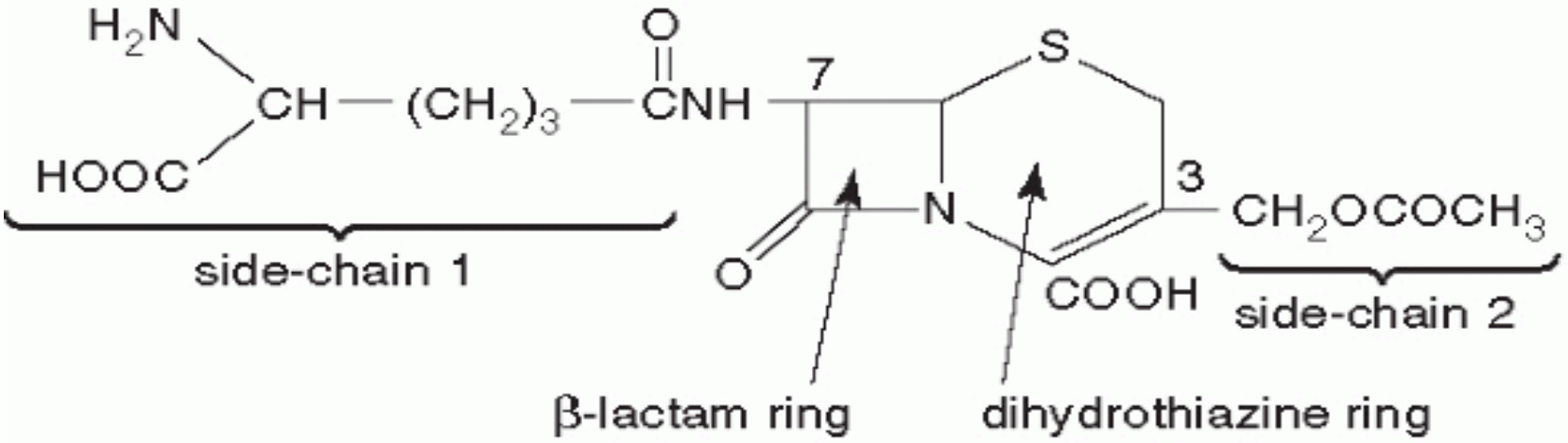
Resistance to penicillin

Some bacteria have the ability to destroy the β-lactam ring of penicillin through the action of bacteria β-lactamase enzyme.

This enzyme is produced by a number of G +ve and G –ve bacteria which have the gene for this enzyme either in plasmid or in chromosomal DNA.



benzylpenicillin



cephalosporin C

Prokaryotic Cell Walls