Lecture 25 & 26: Antimicrobial compounds (Chapter 15)

- Importance of antimicrobial compounds
- Reminder of pre-antibiotic era **LECT 25**
- Bactericidal versus Bacteriostatic
- Antiseptics, Disinfectants,
  - Antiseptic and Disinfectants – mechanism of action resistance ?
- Antibiotics
  - Distribution of drug in body – antiseptic v antibiotics
  - Pharmacokinetics
  - Side effects
  - Antibiotics - characteristics
  - Economics & Process of Antibiotic discovery (see lecture 2 or 3)
  - Antibiotic Targets

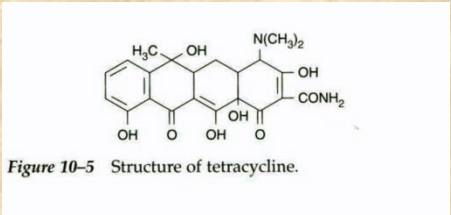


Figure 10–5 Structure of tetracycline.

Cell wall=peptidoglycan layer; pos and neg, Steps, b-lactams; glycopeptides; others

Protein synthesis inhibitors – ribosome structure A sites Aminoglycosides; tetracyclines; macrolides; lincosamide; streptogramins

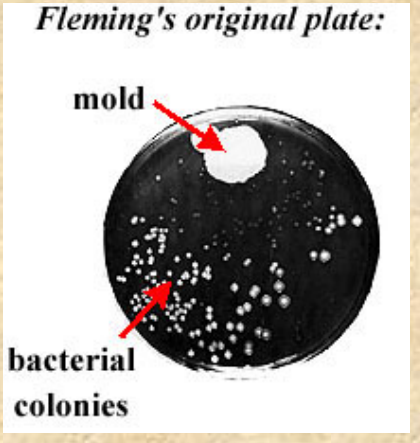
Rifampicin- targeting beta subunit RNA polymerase

DNA replication- DNA gyrase quinolones

Trimethoprin & Sulfonamides – folic acid

Metronidazole- interferes with DNA replication

Newest – include oxazolidones eg. Zyvox like macrolides



Killing versus Inhibiting growth

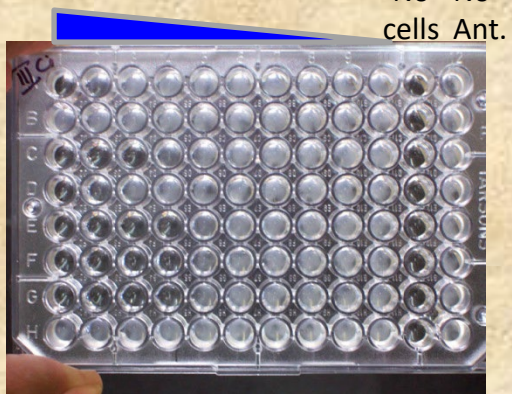
- Destruction (bactericidal) – kill bacteria usually by lysis, although other killing mechanisms exist
- Inhibition (bacteriostatic) – stops or slows the growth of a bacterial population. Useful in patients with intact immune system.
- Importance of knowing your patient
- Where and how the bacteria are growing may determine whether an antibiotic functions as a bactericidal or bacteriostatic compound

Eg 1. Biofilms on catheters

Eg 2. stationary phase or actively growing cells

Susceptibility of bacteria to antimicrobial compounds is measured using simple growth tests, which can be used to calculate:

1) minimal inhibitory concentration (MIC) conc. where bacteria fail to grow; the wells become clear
2) minimal bactericidal concentration (MBC) conc. where bactericidal compounds kill all cells



Antiseptics and Disinfectants

- Antiseptic: kill or inhibit, sufficiently non-toxic so can be applied to living tissues (wounds/handwashing)
- Disinfectants: chemicals that kill microorganisms used in or on inanimate objects
- Most are bactericidal, also usually effective against viruses, fungi and protozoa
- Broad coverage has a drawback – too toxic for internal use
- Antiseptics and Disinfectants tend to attack multiple targets e.g. Halides and bleaches have strong oxidizing power that inactivate proteins
- Quaternary Ammonium compounds (QACs) – e.g. Centrimide, intercalate into phospholipid bilayers causing cells to leak vital ions and other small molecules
- Disinfectants and antiseptics do best against actively replicating cells. Spores are typically resistant to these compounds, although germination can be inhibited.

Common disinfectants and their mode of killing

Disinfectant	**Mode of killing**
Alcohols (ethanol, isopropanol)	Denature proteins
Alkylating agents (formaldehyde, ethylene oxide)	Form epoxide bridges that inactivate proteins
Halides (I^-, Cl^-)	Oxidizing agents
Heavy metals (Hg^{2+}, Ag^+)	Bind –SH groups, thus denaturing proteins
Phenols	Denature proteins, disrupt cell membranes by intercalating in them
Quartenary Ammonium Compounds (QACs)	Disrupt cell membranes by intercalating in them
UV radiation	Blocks DNA replication and transcription by nicking DNA

Resistance to Antiseptics and Disinfectants

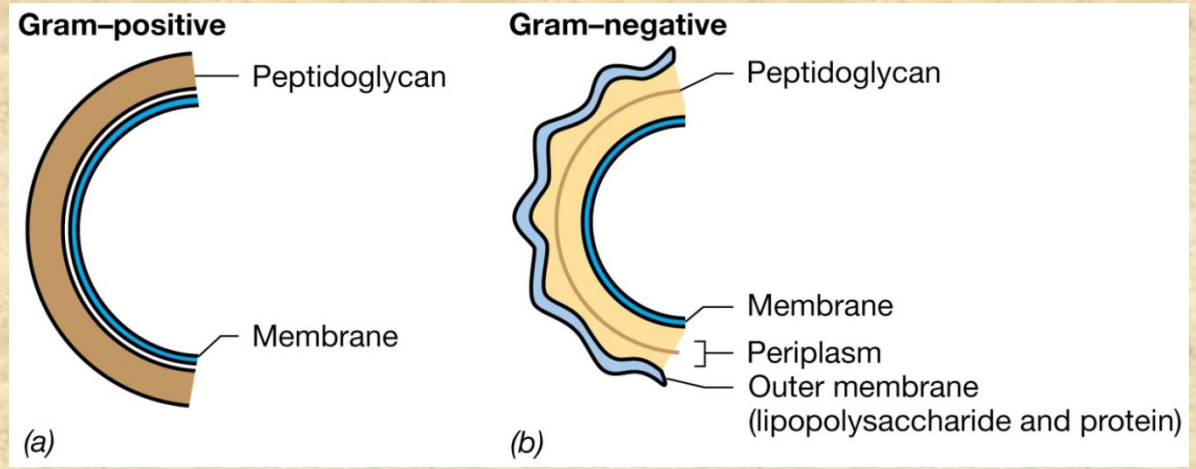
•Misuse is leading to increased bacterial resistance too, although this is generating less media attention. A major concern, however, because these compounds are an important line of defense against microbial infections.

•Resistance to antiseptics and disinfectants is poorly understood, but some mechanisms are known

•<u>General mechanisms</u>

Those antiseptics and disinfectants that attack membranes are more effective against gram positive bacteria. Why? The LPS of gram negative bacteria prevents hydrophobic molecules intercalating with the outer membrane & porins restrict access to cytoplasmic membrane

•<u>Specific mechanisms</u> Staphylococci have been found to pump QACs out of the cytoplasm. Why this makes bacteria resistant to an allegedly membrane dissolving compound is unclear.



Naturally occurring antimicrobial drugs: **Antibiotics**

"Most microbiologists distinguish two groups of antimicrobial agents used in the treatment of infectious disease:

***antibiotics**, which are natural substances produced by certain groups of microorganisms*

***chemotherapeutic agents**, which are chemically synthesized."*

- Less than 1% of antibiotics found in nature are clinically useful
- Many natural antibiotics have been modified in the lab by combinatorial chemistry to enhance their efficacy = **Semisynthetic antibiotics**

*"A hybrid substance is a **semisynthetic antibiotic**, wherein a molecular version produced by the microbe is subsequently modified by the chemist to achieve desired properties. Furthermore, some antimicrobial compounds, originally discovered as products of microorganisms, can be synthesized entirely by chemical means. They might be referred to as **synthetic antibiotics** to distinguish them from the chemotherapeutic agents."*

Source: Todar's Online Textbook of Bacteriology

Antibiotics

- Antibiotics: usually low molecular weight compounds used internally with minimal side effects
- Many antibiotics are made naturally – germ warfare or communication system?
- Classified as bactericidal or bacteriostatic, but also by their specific mode of action (MoA) (i.e the bacterial target molecule)
- Antibiotics typically have a more specific MoA than disinfectants and antiseptics
- All antibiotics are not equal inside the body
- Each class of antibiotic has its own PHARMACOKINETICS (see next slide)

Major groups of microorganisms that produce antibiotics naturally

- The molds – *Penicillium* and *Cephalosporium* produce beta-lactam antibiotics, e.g. penicillin

- Actinomycetes – mainly the *Streptomyces* produce tetracyclines, aminoglycosides, macrolides, chloramphenicol, and most other clinically-useful antibiotics that are not beta-lactams

- *Bacillus* species – *B. polymyxa* and *B. subtilis* produce polypeptide antibiotics (e.g. polymyxin and bacitracin).

Pharmacokinetics

- Definition: describes the distribution of the antimicrobial compound in the body. Also length of residency time in particular tissue before it's removed or degraded

  Examples

  Some antimicrobial compounds used to treat urinary tract infections concentrate primarily in the kidneys and urine and do not disseminate widely in the body

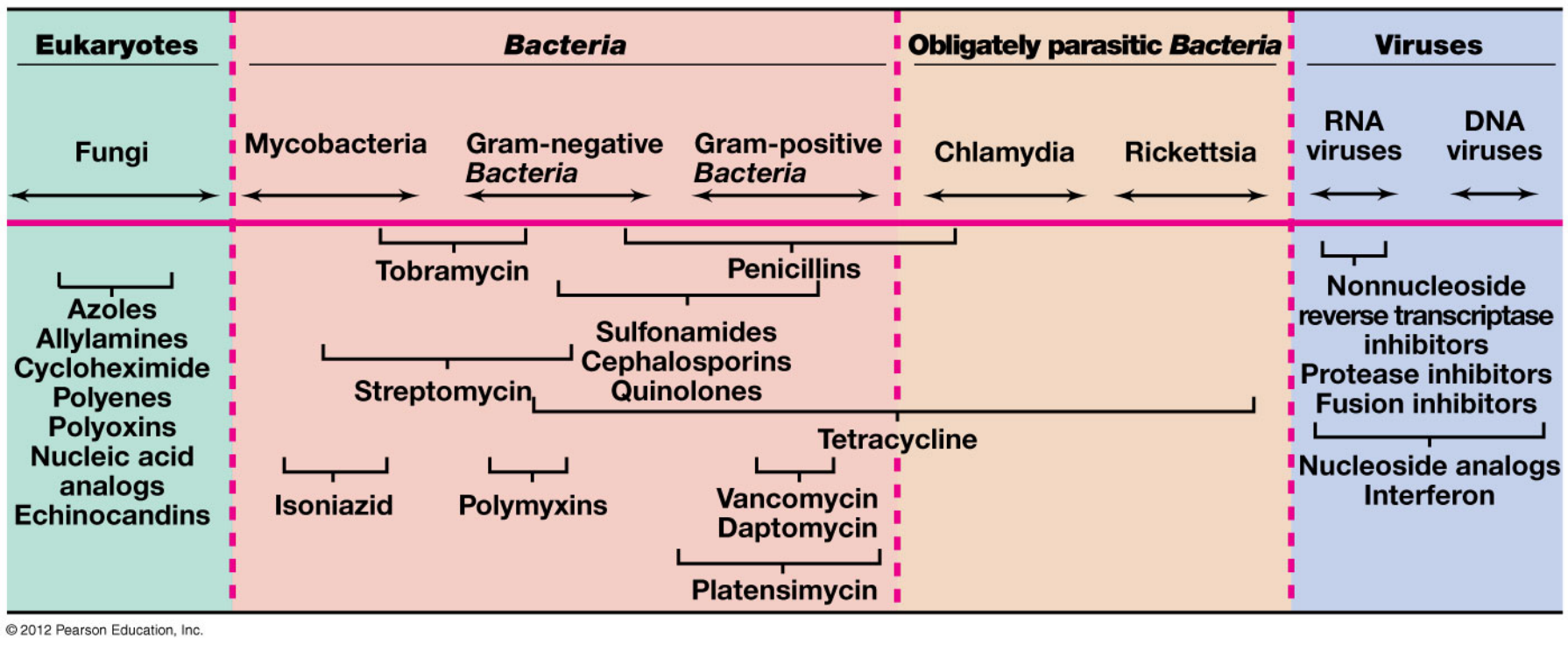
  Other are readily absorbed from the GI tract and permeate tissues throughout the body

  Meningitis is treated with antibiotics that MUST cross the blood-brain barrier

- During drug development, much time is spent determining where the new antibiotic is localized in the human body

What makes a good antibiotic?

- Antibiotic must have few or no side effects = differential toxicity
- Broad spectrum of activity - active against many kinds of bacteria. Why is this important?
- Bioavailability & pharmacokinetics. Bioavailability is a measure of the fraction of the drug or the rate at which a drug that enters the systemic circulation and reaches the site of infection.

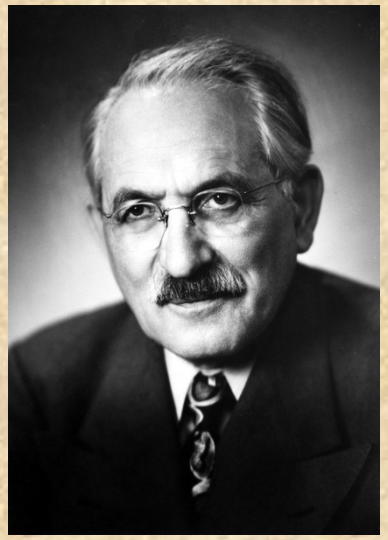


<u>Disadvantages of broad-spectrum antibiotics</u>

microflora considerations, secondary infections, may select for resistance in microflora populations eg. *Enterococcus, Staphylococcus aureus*

Process of antibiotic discovery

Most antibiotics have been obtained from bacteria or fungi that live in the soil



Selman Waksman 1888-1973

Historically
Pharmaceutical companies screened soil isolates for antibiotic producing strains using hypersensitive strains of bacteria

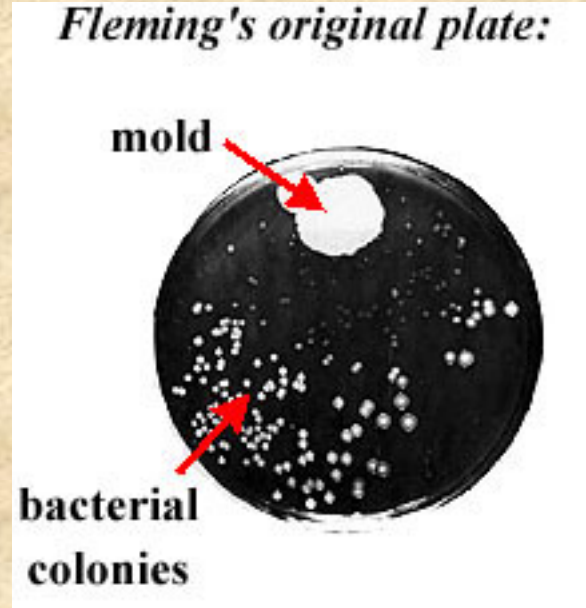
Hypersensitive bacteria were applied as a lawn on an agar plate and various soil isolates were spotted on the plate

The plate was incubated to allow the lawn to become confluent.

Scientist looked for zones of clearing around a particular isolate

Isolates that produces zones of clearance were isolated and the structure of the antibiotic they produced was deduced

Finding bacteria or fungi that produce new kinds of antibiotics is becoming harder and harder



Economic considerations

- A company can spend upto 300 million developing a new antibiotic
- This process can take upto 10-20 years
- At any point, a toxic side effect or lack of efficacy may be uncovered and the company will lose its investment
- <u>Why so expensive and why so time consuming?</u>
- Discovery and characterization
- Animal trials to evaluate efficacy and safety
- Patent applications
- Human clinical trials – small, larger, finally children and pregnant women
- FDA- months / years
- <u>Result:</u>
- In the 1970's, companies started to cut back on their antibiotic discovery programs
- Only in the 1990's was this reversed, may take several more years before we see new antibiotics appearing on the market

Search for new antibiotics

- Structural analogs of previously existing antimicrobial compounds
- New approaches - combinatorial chemistry, bacteriophage-drugs, target identification using crippled bacteria to detect molecules with low level activity that can be built upon eg. Platensimycin, Rational drug design (using structural biology)
- What is Rational drug design? The crystal structure of a target molecule is used to guide the design of chemicals that will bind to the target and inactivate it
- It is estimated that 7 million candidates compound must be screened in order to find one clinically useful drug
- Little economic incentive for drug companies
- Looking in new environments, previously non-culturable organisms http://www.cubist.com/ recently purchased by Merck
- Eg. Daptomycin or cubicin – a lipopeptide antibiotic
- Teixobactin - Kim Lewis, Northeastern University Nature paper 2015 (link on Webcampus)

What makes a good target for antibiotic therapy?

Unique; Bacteria v Eukaryote
Widespread

Hypomutatable?

Targets of Antibiotic Action

- The susceptibility of microorganisms to individual antibiotics vary significantly
- Broad spectrum antibiotics v Narrow spectrum antibiotics
- There are many different targets of antibiotics (next slides)

Cell Wall Synthesis inhibitors

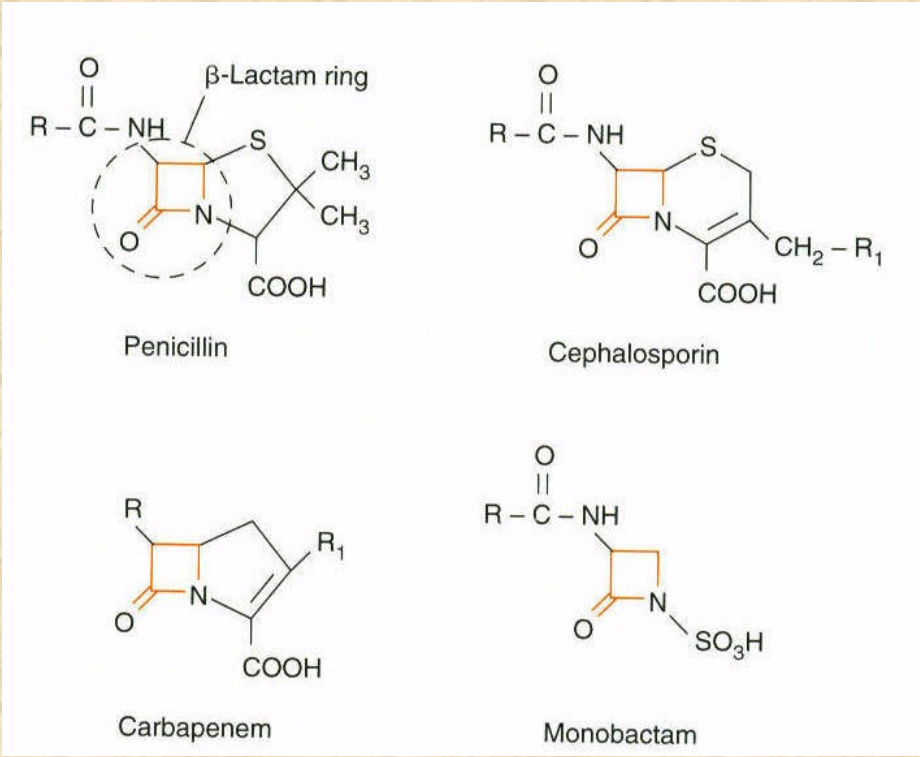
β-lactam antibiotics

Four-membered ring common to all

Among the most useful, however..

Main problem: allergic reaction caused by β-lactam/serum protein conjugate which evokes an immune response

Monobactams don't cause this allergy



β-lactams target the transpeptidation reaction

β-lactams also bind and inhibit inner membrane enzymes that carry out peptidoglycan synthesis

AKA Penicillin-binding proteins

Usually bactericidal - resulting in cell lysis

Some β-lactams are effective against both gram-positives and gram-negatives.

Others are more effective against one or the other

Vary widely in their toxicity, stability in the body, clearance from the blood, whether they can be taken orally and their ability to cross blood-brain barrier

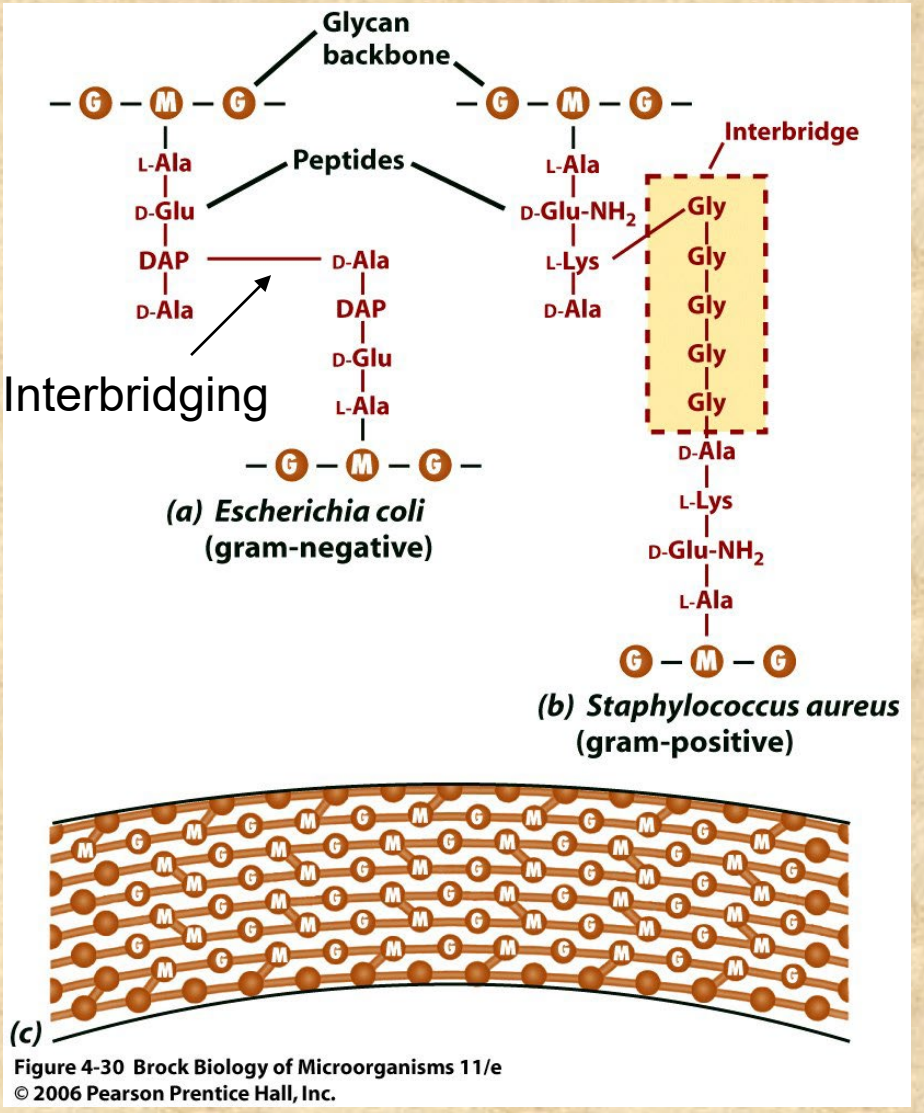


Figure 4-30 Brock Biology of Microorganisms 11/e

Other Antibiotics that target peptidoglycan synthesis

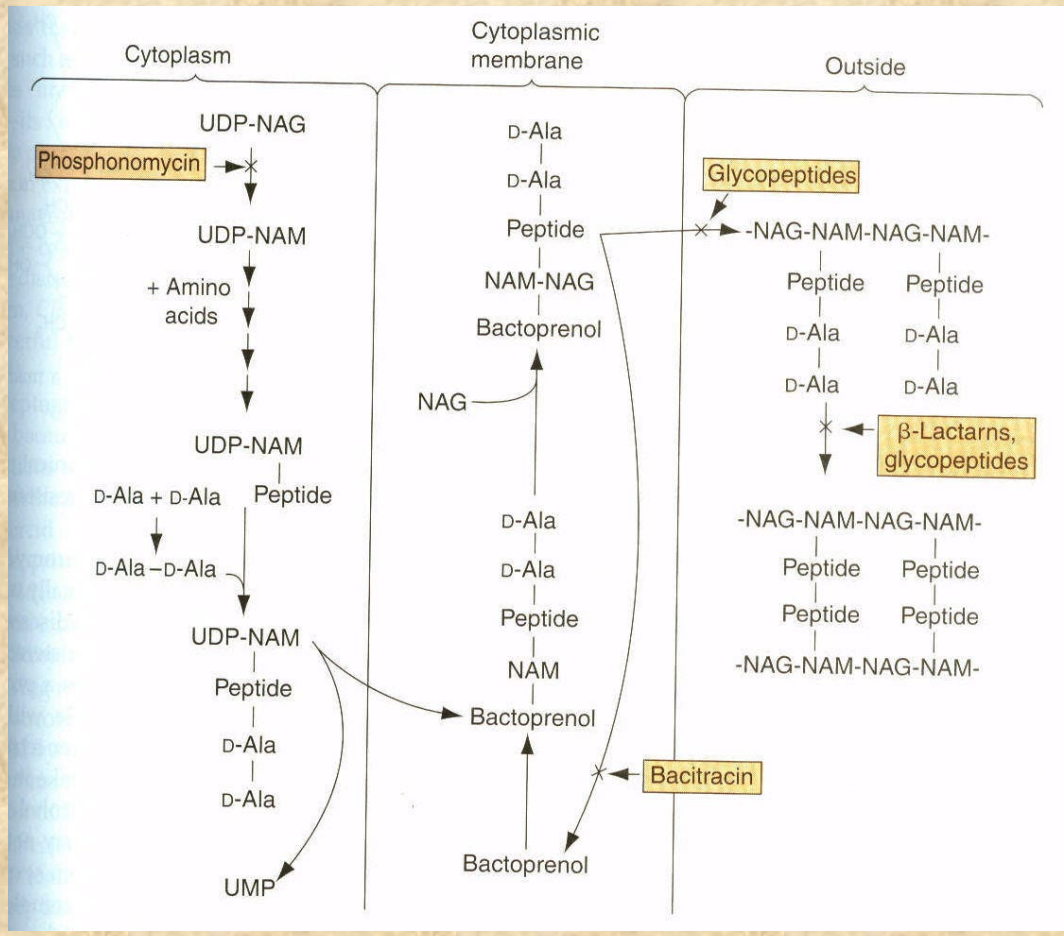
•<u>Glycopeptides</u>
Eg. Vancomycin, Daptomycin (cubist) and Teicoplanin.
Vancomycin is the last drugs effective against antibiotic resistant gram-positive pathogens
eg. MRSA & *Enterococci*

Binding appears to inhibit both transglycosylation and transpeptidation

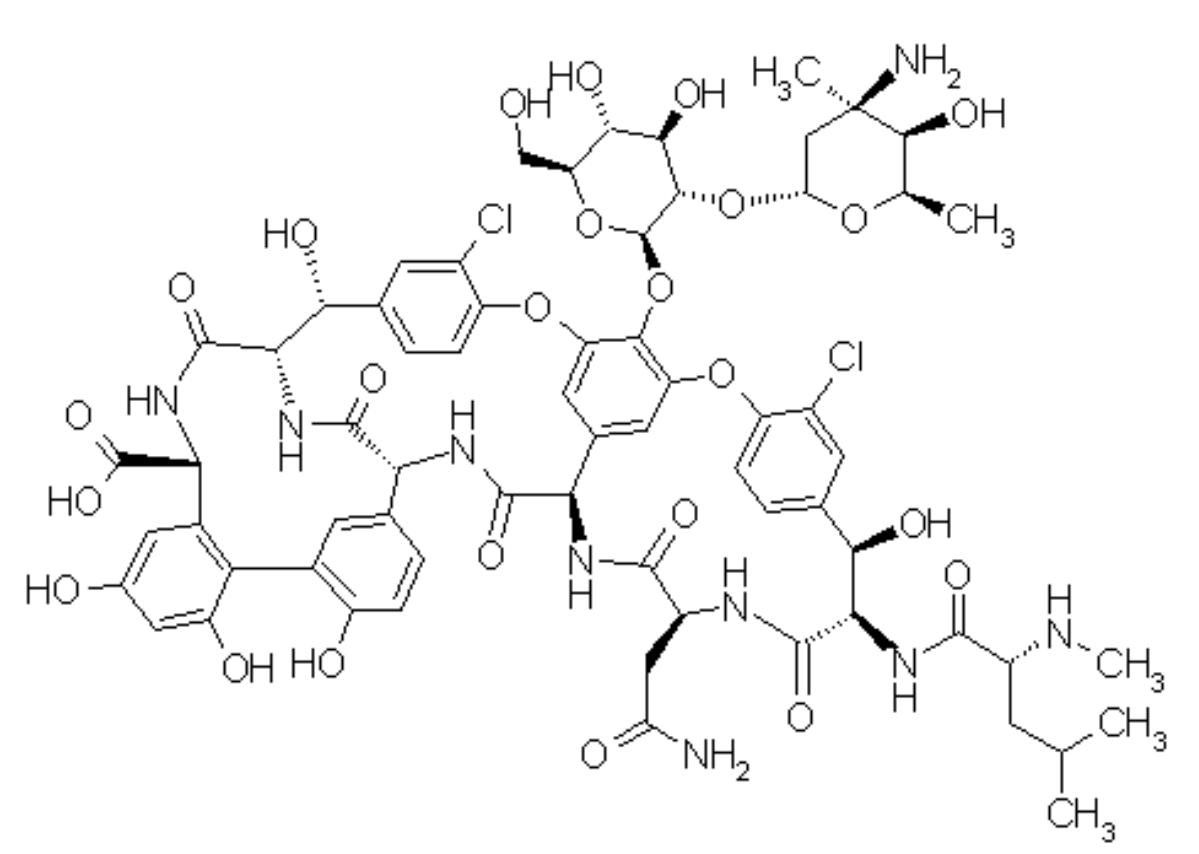
Not very effective against gram-negative bacteria – narrow spectrum

<u>Others</u>
Eg. Phosphonomycin and Bacitracin
Inhibit early steps in the cell wall synthesis
Phosphonomycin (aka Fosfomycin) works against MRSA
Bacitracin is too toxic for internal use frequently found in topical creams

Steps in synthesis of peptidoglycan and the effects of different classes of antibiotic



Structure of Vancomycin – a glycopeptide, naturally occurring antibiotic



…one of the last effective drugs against MRSA. Once administered by injection, now oral version available.

Protein synthesis inhibitors

Aminoglycosides

Eg. Streptomycin, Kanamycin (tobramycin) and Gentamicin [derived from bacterial Genera *Streptomyces* –mycin; *Micromonospora* -micin]

Target the bacterial ribosome, specifically binding the 30S subunit and preventing interaction with the 50S subunit. Prevents ribosome complex formation, so no protein synthesis occurs

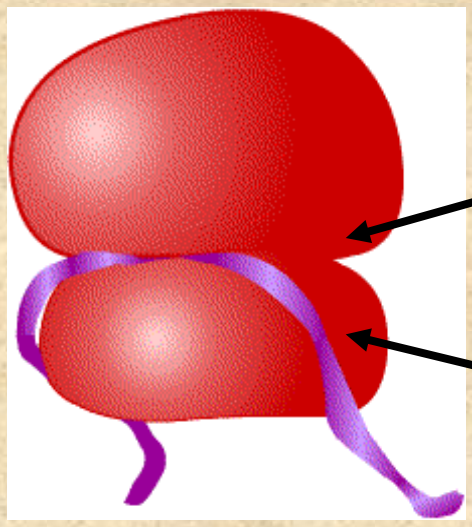
Also reported to cause bacterial membrane blebbing, explaining the bactericidal activity (requires LPS)

Not absorbed by the gut, typically administered intravenously and intramuscularly.

Have side effects at high doses that limit their use - hearing loss and impairment of kidney function

Most effective against aerobic, gram negative & Mycobacteria, including *M.tb*. Less effective against anaerobic bacteria

Ribosomes: the translation factory



50S subunit
= 5S rRNA + 23S rRNA + ~34 proteins

30S subunit
= 16S rRNA + ~21 proteins

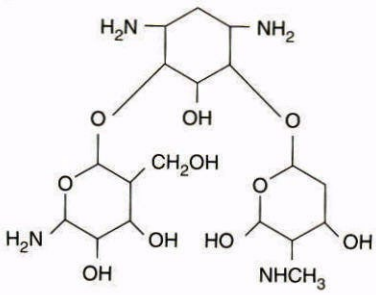


Figure 10–4 Structure of gentamicin, an aminoglycoside.

Aminoglycosides=trisaccharides with amino groups

Tetracyclines

Four fused cyclic rings

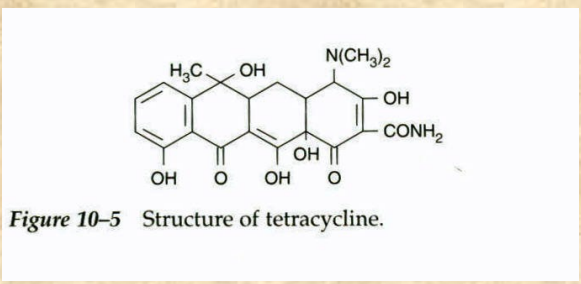


Figure 10–5 Structure of tetracycline.

Target the bacterial ribosome and bind the 30S subunit

Distort the A site and prevents the alignment of tRNA with the codon in the mRNA

Newest tetracycline is glycyl-glycine-tetracycline (aka tigecycline) – bulky side chain. Approved for use against gram negative and positive pathogens

Tetracyclines generally bacteriostatic

Least toxic antibiotics available (although some discoloration of teeth in young children). Broad spectrum given orally - widespread use – resistance common

Ribosomes: the translation factory