ANTIBIOTICS

III.

- An important year in chemotherapy of systemic bacterial infection was 1935.
- Although antiseptics had been applied topically to prevent growth of microorganisms, systemic bacterial infections did not respond to any existing agents.
- In 1935 the red azodye **prontosil** was shown to protect mice against systemic streptococcal infection and was curative in patients suffering from such infections.

- Eventually, compounds (antibiotics) produced by microorganisms were discovered to inhibit the growth of other microorganisms.
- Fleming first noted that the mold *Penicillium* prevented the multiplication of staphylococci. A concentrate from a culture of this mold was prepared, and remarkable activity and lack of toxicity of the first antibiotic, penicillin, was demonstrated.
- Later, in the 1940s and 1950s, streptomycin and the tetracyclines were developed and were followed rapidly by additional aminoglycosides, semisynthetic penicillins, cephalosporins, quinolones and other antimicrobials.

The basic mechanisms of antibiotic action

- **Inhibition of cell wall synthesis**
  - The cross - linkage of precursors during synthesis of the bacterial cell wall is catalyzed by specific enzymes.
  - These enzymes are called Penicillin Binding Proteins (PBPs).
  - The rigid structure of the cell wall permits bacteria to maintain a very high internal osmotic pressure. However, when bacteria are exposed to penicillin and the antibiotic binds to the PBPs in the cell wall, autolytic enzymes are released that degrade the preformed cell wall.

The basic mechanisms of antibiotic action

- **Alteration of cell membranes**
  - The polymyxin class of antibiotics consists of cationic branched cyclic decapeptides, that destroy the cytoplasmatic membranes of susceptible bacteria.
  - The antifungal polyene antimycotics (e.g. amphotericin B, nystatin) have a similar activity on cell membranes.

The basic mechanisms of antibiotic action

- **Inhibition of protein synthesis**
  - Antibiotics such as tetracyclines, macrolides, chloramphenicol and aminoglycosides inhibit protein synthesis.
  - After the antibiotics enter the cell and transverse the cell membrane, they bind to ribosomal subunits.
  - Some antibiotics inhibit mitochondrial proteins synthesis, others stop elongation of nascent protein, and the action of other antibiotics leads to deformation of proteins.

The basic mechanisms of antibiotic action

- **Inhibition of nucleic acid synthesis**
  - Some antibiotic agents inhibit nucleic acid synthesis by either binding to RNA polymerase (e.g. rifampicin) or inhibiting DNA gyrase (e.g. quinolones).

The basic mechanisms of antibiotic action

- **Antimetabolic activity or competetive antagonism**
  - Some antibacterial compounds act as antimetabolites.
  - Sulphonamide competes with para-aminobenzoic acid, preventing synthesis of folic acid that is required by certain microorganisms. Because mammalian organisms do not synthesis folic acid, sulphonamides do not interface with mammalian cell metabolisms.

Beta-lactam antibiotics

- This is a large family of different groups of compounds all containing the beta-lactam ring.
- The different groups within family are distinguished by the structure of the ring attached to the beta-lactam ring; in penicillins this is a five-membered ring, in cephalosporins a six-membered ring, and the side chains attached to these ring.
- These antibiotics inhibit cell wall synthesis.

- ***Absorption, distribution and excretion***
  - The majority of beta-lactams have to be administered intramuscularly or intravenously, but there are also orally active agents.
  - Most achieve clinically useful concentrations in the CSF when the meninges are inflamed (as in meningitis) and the blood-brain barrier become more permeable.
  - In general, they are not effective against intracellular microorganisms.
  - Beta-lactams are excreted mostly in the urine.

- ***Uses***
  - There are more than 60 different beta-lactam antibiotics currently registered for clinical use. Some, such as penicillin, are active mainly against grampositive microorganisms, whereas other have been developed for their activity against gramnegative rods such as the enterobacteria.

- ***Resistance***
  - Clinical isolates resistant to beta-lactams may exhibit any one (or more than one) of three mechanisms of resistance:
    - alteration in target site,
    - alternation in access to the target site,
    - production of beta-lactamases.

β-Lactams

The four classes of β-lactams

1940	Penicillins
1960	Cephalosporins
1970	Monobactams
1980	Carbapenems

Spectrum of β-lactams

- Staphylococci, streptococci, some enterococci
- *Enterobacteriaceae*
- *Pseudomonas* spp.
- Anaerobes – gramnegative and grampositive
- *Neisseria* spp., *Haemophilus* spp.

Beta-lactam antibiotics

- **Penicillins**
  - Basic:
    - benzylpenicillin
    - phenoxymethylpenicillin
  - Aminopenicillins
    - ampicillin
    - amoxicillin
  - Carboxypenicillins (antipseudomonal drugs)
    - tikarcillin
  - Ureidopenicillins (antipseudomonal drugs)
    - azlocillin
    - piperacillin

Bacteria remaining fully sensitive to benzylpenicillin

- Group A β-haemolytic streptococci
- *Neisseria meningitidis*
- Most *Clostridia* species
- Anaerobic cocci
- Spirochaetes including *Treponema pallidum*

The problems of benzylpenicillin

- Difficulty in penetration of the outer membrane of gramnegative rods

  Mainly solved by newer penicillins and newer cephalosporins

- Loss of affinity to penicillin-binding proteins

  Partly solved by higher doses and combined therapy

- Beta-lactamases

Beta-lactam antibiotics - penicillins

- Resistant to staphylococcal penicillinase
  - oxacillin
  - methicillin
- Combination with inhibitors of bacterial beta-lactamases
  - ampicillin/sulbactam
  - amoxicillin/clavulanic acid
  - piperacillin/tazobactam

Beta-lactam antibiotics

- **Cephalosporins**
  - Cephalosporins of I. generation
    - cephalotin, cefazoline, cefalexin, cefaclor, cefadroxil
  - Cephalosporins of II. generation
    - cefuroxime, cefoxitin, cefprozil, cefuroxime/axetil
  - Cephalosporins of III. generation
    - cefotaxime, ceftriaxone, ceftazidime, cefoperazone,
  - Cephalosporins of IV. generation
    - cefepime

Types of bacteria with main resistance mechanisms

Staphylococci	enzymes, PBPs
Other grampositive cocci	PBPs
Enterobacteria	enzymes, penetration,
Pseudomonas	enzymes, penetration, PBPs
Anaerobes	enzymes

Antibiotic strategies for antibiotic-resistant bacteria containing β-lactamases

a) Use of beta-lactamase-stable compounds
new cephalosporins; carbapenems

b) Use of beta-lactamase inhibitors
sulbactam; tazobactam; clavulanic acid

c) Use of non-beta-lactam compounds
aminoglycosides; quinolones

Beta-lactam antibiotics

- **Monobactams**
  - aztreonam
- **Carbapenems**
  - imipenem
  - meropenem
  - ertapenem
  - doripenem

Aminoglycosides

- Aminoglycosides inhibit and kill microbes by interfering with protein synthesis.
- *Absorption, distribution and excretion*
  - Aminoglycosides are not absorbed from the gut and must be given intravenously or intramuscularly for systemic treatment.
  - They do not penetrate well into tissues and bone and do not cross the blood-brain barrier.
  - Intrathecal administration of streptomycin is used in treatment of tuberculous meningitis, and gentamicin may be administered by this route for the treatment of gramnegative meningitis in neonates.
  - Aminoglycosides are excreted via the kidney.

Aminoglycosides

- ***Uses***
  - Gentamicin and the newer aminoglycosides, tobramycin, amikacin, netilmicin and isepamicin are important for the treatment of serious gramnegative infections including those caused by *Pseudomonas aeruginosa* strains.
  - They are not active against streptococci, but they have activity against staphylococci.
  - They are not active against anaerobes.
- ***Toxicity***
  - The aminoglycosides are potentially nephrotoxic and ototoxic, and the terapeutic "window" between serum concentrations required for succesful treatment and those that are toxic, is small. Blood concentration should be monitored regularly, particularly in patients with renal impairment. Netilmicin and isepamicin are reported to be of lower toxicity than the other aminoglycosides.

Aminoglycosides

- ***Resistance***
  - Resistance may arise in gram-negative rods through alternation in cell wall permeability.
  - Production of aminoglycoside-modifying enzymes is the most important mechanism of acquired resistance.

Aminoglycosides

Streptomycin

Neomycin

Gentamicin

Netilmicin

Tobramycin

Isepamicin

Amikacin

Aminoglycosides

- Once-daily administration is advocated to maximise efficacy and minimise potential drug accumulation and toxicity

Moore et al. J Infect Dis 1987;155:93–99
Kashuba et al. Antimicrob Agents Chemother 1999;43:623–629
Nicolau et al. Antimicrob Agents Chemother 1995;39:650–655

Tetracyclines

- Tetracyclines inhibit protein synthesis.
- These drugs are usually administered orally.
  - **Doxycycline** and **minocycline** are more completely absorbed than **tetracycline** and **oxytetracycline**, resulting in higher serum concentration and less gastrointestinial upset because there is less inhibition of normal gut microflora.
- Tetracyclines are used for treatment of infection caused by mycoplasmas, chlamydiae and rickettsiae.
- Tetracyclines are active against a wide variety of different bacterial species, but their use is now restricted by widespread resistance.

Tetracyclines

- ***Toxicity***
  - Suppresion of normal gut flora causes gastrointestinal upset, diarrhoea and encourages overgrowth by resistant and undesirable bacteria (e.g. *Staphylococcus aureus*) and fungi (e.g. *Candida* spp.).
  - Interference with bone devolopment and brown staining of teeth occurs in the foetus and in children and thus these drugs should be avoid in pregnancy and in children under 8 years of age.
  - Systemic administration may cause liver damage.
- Resistance is common, due partly to the widespread use of these drugs in humans and also to their use as growth promoters in animal feedstuffs.

Chloramphenicol

- The drug is well absorbed when given orally, but can be given intravenously if the patient cannot take drug by mouth.
- Topical drugs are also available.
- It is well distributed in the body and penetrates host cells.
- Chloramphenicol is metabolised in the liver by conjugation with glucuronic acid to yield a microbiologically inactive form that is excreted by the kidneys.

Chloramphenicol

- ***Uses***
  - Chloramphenicol is active against a wide variety of bacteria species both grampositive and gramnegative, aerobes and anaerobes, including intracellular microorganisms such chlamydiae and rickettsiae.
  - It achieves satisfactory concentrations in the cerebrospinal fluid and is valuable in the treatment of bacterial meningitis.
  - Topical drugs are used for eye infections.

Chloramphenicol

- ***Toxicity***
  - It is rare but serious toxic effects of chloramphenicol that have tended to restrict use of this drug in countries where alternative agents are readily available.
  - The most important toxic effects are in the bone marrow. This toxicity has two forms:
    - dose-dependent bone marrow suppression, which occurs if the drug is given for long periods and is reversible when treatment is stopped,
    - an idiosyncratic reaction causing aplastic anemia, this is not dose-dependent and is irreversible. It can occurs after treatment has stopped but is fortunately very rare, occurring in about 1 in 30 000 patients treated.
  - Chloramphenicol is also toxic to neonates particularly premature babies whose liver enzyme systems are incompletely developed. This can result "gray baby syndrome".

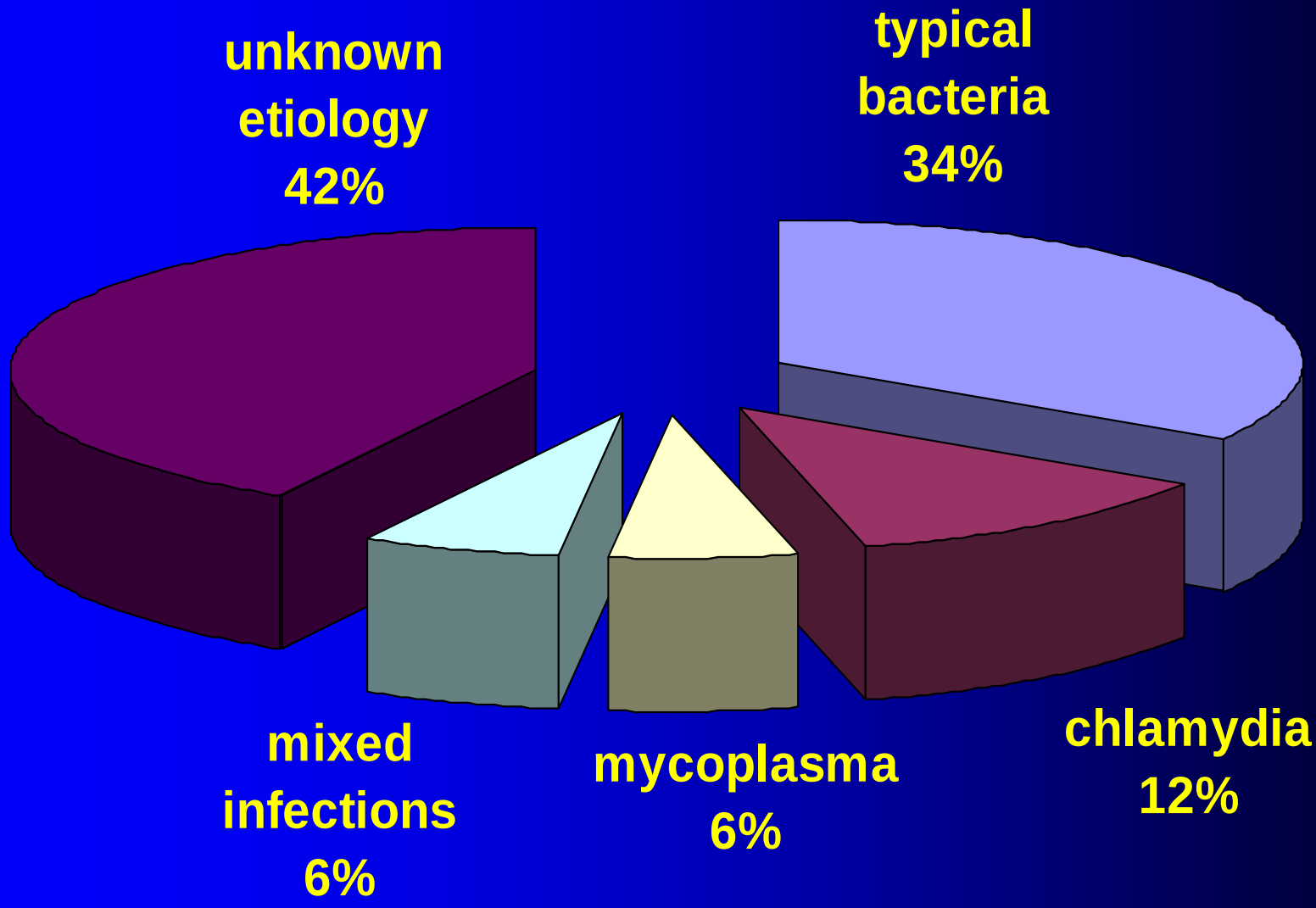
Macrolides

- **Erythromycin** is the best known but some of the newer agents such **roxithromycin**, **clarithromycin** and **azithromycin**, with improved activity and pharmacology, may take its place for many indications. **Spiramycin** is another macrolide used for the treatment of toxoplasmosis.
- Macrolides are usually administered by the oral route but can also be given intravenously.
- These drugs are well distributed in the body and penetrate mammalian cells to reach intracellular microorganisms.
- They are concentrated in the liver and excreted in the bile. A small proportion of the dose is recoverable in the urine.

Macrolides

- ***Uses***
  - Macrolides are active against grampositive cocci and they are an important alternative treatment of infections caused by streptococci in patients allergic to penicillin.
  - They are active against *Legionella pneumophila* and *Campylobacter* spp. strains.
  - They are also active against mycoplasmas, chlamydiae and rickettsiae and therefore can be considered as important drug for treatment of atypical pneumonia and chlamydial infections of the urogenital tract.
- ***Toxicity***
  - Macrolides are relatively non-toxic drugs although they cause nausea and vomiting after oral administration in a significant number of patients. Jaundice is associated with some formulations of the drug.

Etiology of community-acquired pneumonia in olomouc region



According the data from olomouc region it is possible to make a conclusion:

- **CAP**
  - typical pneumonias form about 65 %
  - atypical pneumonias form about 35 %
    - *chlamydia pneumonias* 24 %
    - *mycoplasma pneumonias* 11 %

Lincosamides

- The two important drugs in this group are **lincomycin** and **clindamycin**. In the present, clindamycin is more used than lincomycin.
- Lincosamides are given orally, intramuscularly and intravenously.
  - The drugs penetrate well into bone but not into cerebrospinal fluid even when the meninges are inflamed.
  - They are actively transported into polymorphonuclear leucocytes and macrophages.
  - They are metabolised in the liver to several products with variable antibacterial activity and lincosamides activity persists in faeces for up to days after a dose.

Lincosamides

- ***Uses***
  - Lincosamides have a spectrum of activity similar to macrolides but they are much more active against anaerobes, both grampositive e.g. *Clostridium* spp., and gramnegative e.g. *Bacteroides* spp.
  - However, *Clostridium difficile* is resistant and may be selected in the gut, causing pseudomembranous enterocolitis.
  - The activity of lincosamides against *Staphylococcus aureus* and their penetration into bone make them valuable drugs in the treatment of osteomyelitis.
- ***Toxicity***
  - The association between antibotic administration and pseudomembranous enterocolitis caused by *Clostridium difficile* was first noted following clindamycin treatment but has been shown to follow treatment with many wide-spectrum antibiotics. Oral vancomycin or metronidazol should be used to treat pseudomembranous enterocolitis.

Glycopeptides

- Vancomycin
- Teicoplanin
- ***Uses***
  - Glycopeptides are active against multiresistant grampositive cocci and they are an important treatment of infections caused by MRSA or MRSCN.
  - Oral vancomycin is indicated in antibiotic-associated pseudomembranous enterocolitis.

Sulphonamides

- Sulphonamides are usually administered orally often in combination with trimethoprim as cotrimoxazole (sulphamethoxazol+trimethoprim).
- The sulphonamides have a spectrum of activity primarly against gramnegative bacteria (except *Pseudomonas* spp.) and can be active against grampositive bacteria. Thus they are useful in the treatment of urinary tract infection.
- However resistance is widespread and susceptibility cannot be assumed.
- ***Toxicity***
  - They are relatively free of toxic side effects but rashes and bone marrow suppresion can occur.

Quinolones

- This is a large family. Nalidixic acid and oxolinic acid are ones of the earlier protypes, but the synthesis of fluoroquinolones has led to an enormous number of chemical derivates with improved antibacterial activity.

- Quinolones are inhibitors of DNA replication.

- Quinolones act by inhibiting the activity of DNA gyrase and thereby preventing supercoiling of the bacterial chromosome. As a result the bacterial cell can no longer "pack" its DNA into the cell. The inhibition is specific to bacterial gyrase and does not affect the equivalent topoisomerase in mammalian cells.

Quinolones

- Quinolones are administered orally and parenterally, are well-absorbed from the gastro-intestinal tract and are excreted mostly in the urine but a small proportion in the faeces.
- Nalidixic and oxolinic acid do not achieve adequate serum concentration for systematic therapy, but the newer fluoroquinolones achieve significant serum concentration after oral dosage and are very well-distributed throughout the body compartments.
- Fluoroquinolones:
  pefloxacin, ofloxacin, ciprofloxacin, levofloxacin, norfloxacin, moxifloxacin, gatifloxacin and others

Quinolones

- ***Uses***
  - **Nalidixic and oxolinic acid** are active only against enterobacteria and the use is confined to the treatment of urinary tract infection.
  - The newer quinolones such as **ofloxacin, pefloxacin, norfloxacin, ciprofloxacin, levofloxacin** etc. have a greater degree of activity against gramnegative rods. They are active also against *Pseudomonas aeruginosa*.
  - In addition to the treatment of urinary tract infection the newer quinolones are useful for systematic gramnegative infections and may find a role in the treatment of chlamydial and rickettsial infections.
  - They may also be useful in infections caused by *Legionella pneumophila*, *Salmonella typhi* and in combination with other agents for "atypical" mycobacteria.
  - They have activity against staphylococci but less against streptococci.

Quinolones

- ***Toxicity***
  - Gastro-intestinal disturbances are the most common side effects. Neurotoxicity and photosensitivity reactions occur in 1-2% of patients. The fluoroquinolones are not licensed at present for use in children because of possible toxic effects on cartilage development.
- ***Resistance***
  - Chromosomally mediated resistance occurs and is exhibited in two forms:
    - changes in DNA gyrase subunit structure resulting in a lowered afinity for the drug,
    - changes in cell wall permeability, resulting in decreased uptake, this mechanism may also lead to cross resistance to other unrelated agents taken up by the same route

Inhibitors of cytoplasmic membrane function

- The most important from these antimicrobial agents are the polymyxins which act on the membranes of gram-negative bacteria.
- The polyene antifungal agents (amphotericin B, nystatin) also act by inhibiting membrane function.

Polymyxins

- A group of polypeptide antibiotics that consists of 5 chemically different compounds (polymyxins A-E), were discovered in 1947
- Only polymyxin B and **polymyxin E (colistin)** have been used in clinical practice
- The primary route of excretion is renal

Polymyxins

- Colistin (polymyxin E) is the most common member of the family in clinical use
- Colistin is active against gramnegative microorganisms except *Proteus* spp., *Morganella* spp. and *Providencia* spp.
- As an oral agent it is used in some gut decontamination regimenes for neutropenic patients and in colorectal surgery
- ***Toxicity***
  - Colistin is nephrotoxic

Colistin

- The target of antimicrobial activity of colistin is the bacterial cell membrane
- Colistin has also potent anti-endotoxin activity
  - The endotoxin of G-N bacteria is the lipid A portion of LPS molecules, and colistin binds and neutralizes LPS

Colistin

- Active:
  - *Acinetobacter* species,
  - *Pseudomonas aeruginosa*,
  - *Enterobacteriaciae*