ANTIBIOTICS

Faculty of Dentistry

22 September 2014

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Structure of the lecture

- History of antibiotics
- Principles of antibiotic treatment
- Mode of actions of antibiotics
- Resistance to antibiotics

- Determination of antibiotic sensitivity

HISTORY OF ANTIBIOTICS

History of antibiotics - 1

- 19th century:
  - Louis Pasteur & Robert Koch:

Bacteria as causative agents & recognized need to control them

History of antibiotics - 2

- Plant extracts
  - Quinine (against malaria)
  - Ipecacuanha root (emetic, e.g. in dysentery)
- Toxic metals
  - Mercury (against syphilis)
  - Arsenic (Atoxyl, against Trypanosoma)
- Dyes
  - Trypan Blue (Ehrlich)
  - Prontosil (azo-dye, Domagk, 1936)

History of antibiotics - 3

Paul Ehrlich

- started science of chemotherapy
- systematic chemical modifications
  ("Magic Bullet")
  no. 606 compound = **Salvarsan** (1910)
- selective toxicity !!
- developed the **Chemotherapeutic Index**

$$\text{Chemotherapeutic Index} = \frac{\text{Toxic Concentration}}{\text{Effective Concentration}}$$

Chemotherapeutic index

$$\frac{\text{DTM}}{\text{DCM}}$$ the larger, the better

- DTM = dosis tolerata maxima (toxic)
- DCM = dosis curativa minima (effective)

- wide or narrow application concentration interval

History of antibiotics - 4

Penicillin- the first antibiotic - 1928

- **Alexander Fleming** observed the killing of staphylococci by a fungus (*Penicillium notatum*)
- observed by others - never exploited
- Florey & Chain purified it by freeze-drying (1940) - Nobel prize 1945
- first used in a patient: 1942
- World War II: penicillin saved 12-15% of lives

Fleming Museum, London

History of antibiotics - 5

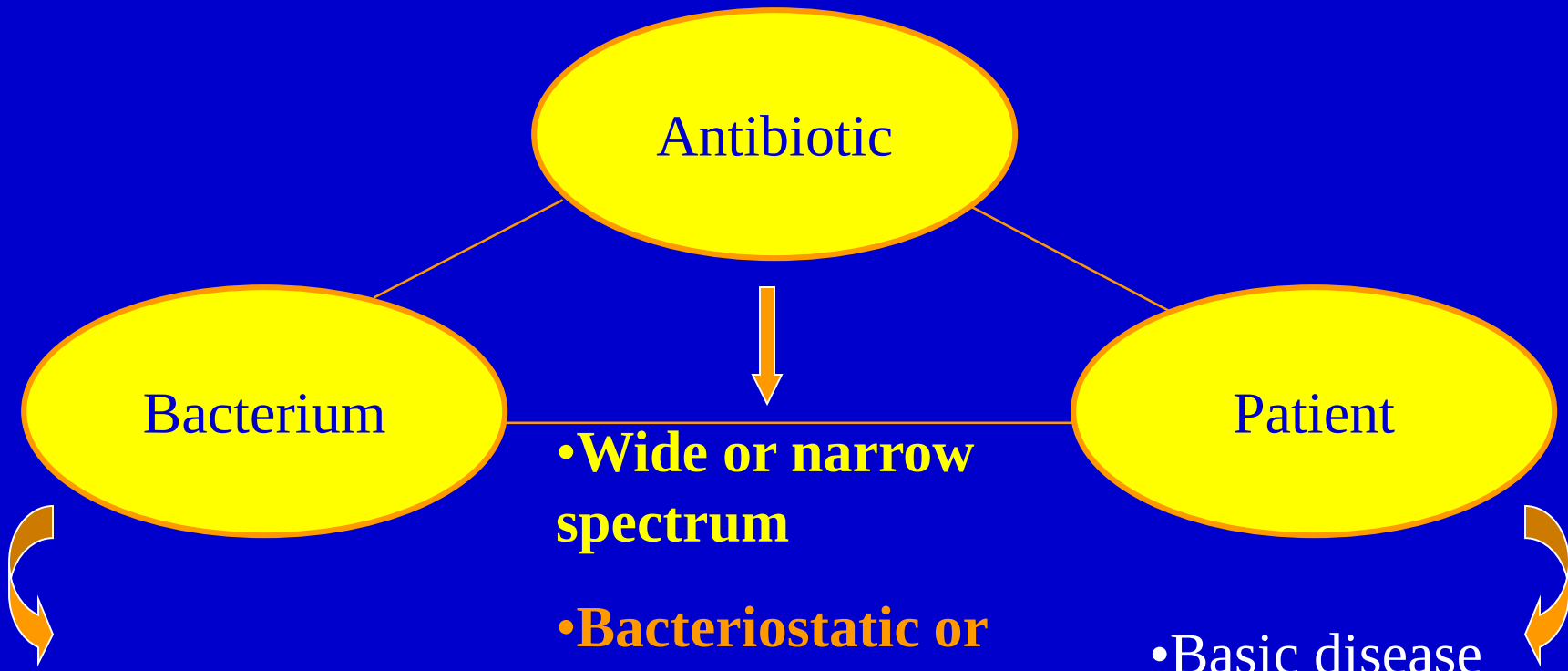
- Selman Waksman - Streptomycin (1943)
  - active against all **Gram-negatives**
  - **first antibiotic active against *Mycobacterium tuberculosis***
  - most severe infections were caused by Gram-negatives and *Mycobacterium tuberculosis*
  - extracted from *Streptomyces*
  - 20 other antibiotics, incl. neomycin, actinomycin

Nobel prize

1952

PRINCIPLES OF ANTIBIOTIC TREATMENT

Principals of antibiotic treatment



Types of antibiotic therapy

- Targeted
  - based on sensitivity tests
- Empiric
  - based on the symptoms and habits
  - knowledge of local epidemiological data
- Profilactic
  - e.g. intestinal operation, dentical surgery

Possible side effects

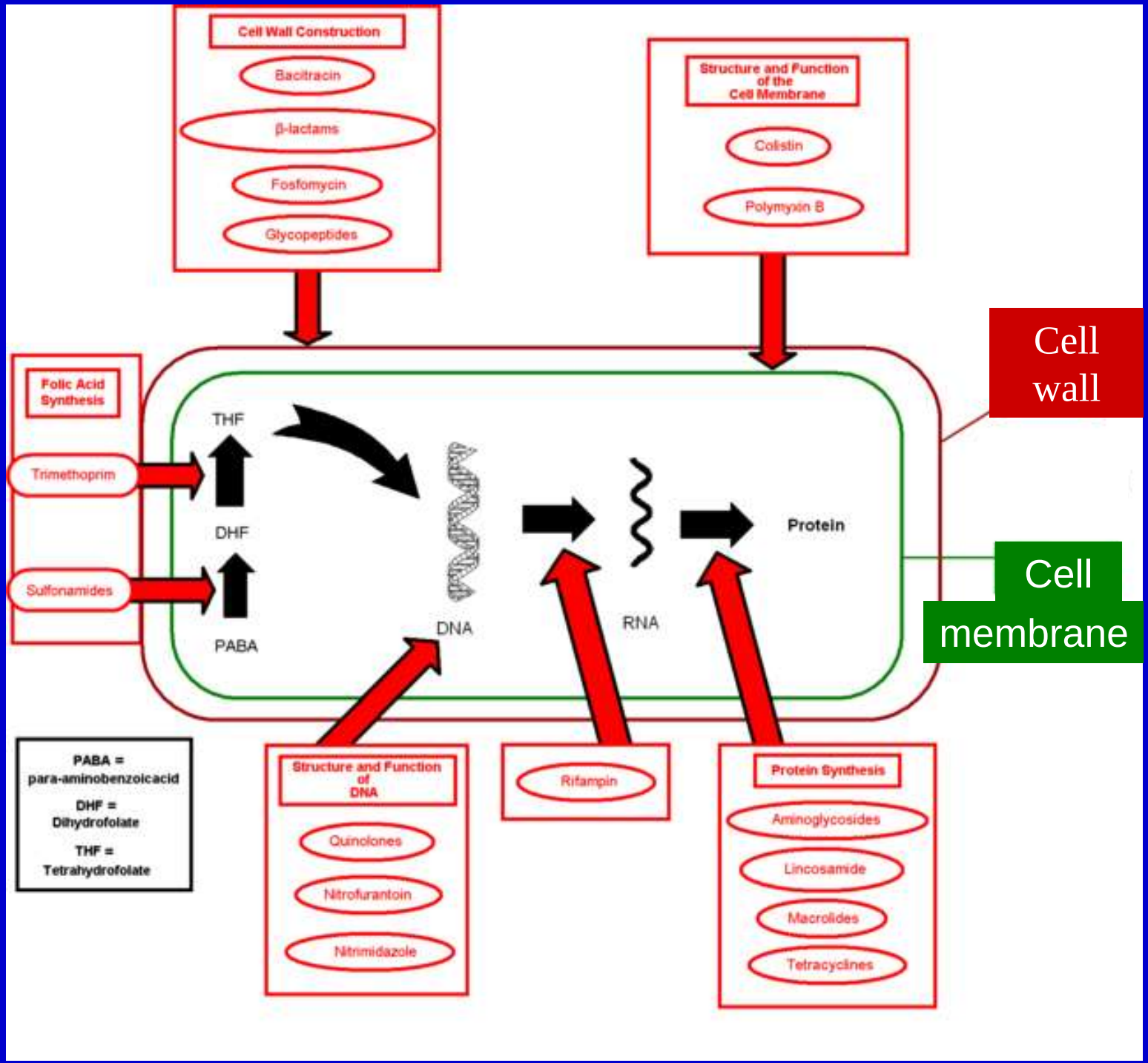
- Allergy
  - penicillins!
  - type I hypersensitivity reaction (anaphylaxy)
- Toxic effect
  - kidney, liver (alcoholism!), bone marrow
  - impaired hearing
  - bones, teeth (tetracyclin: complex with Ca^{2+})
  - fluoroquinolones: Achilles-tendon rupture
- Disbacteriosis
  - = killing of the normal flora
  - e.g. pseudomembranous colitis by *C. difficile*

MODE OF ACTIONS OF ANTIBIOTICS

Possible targets

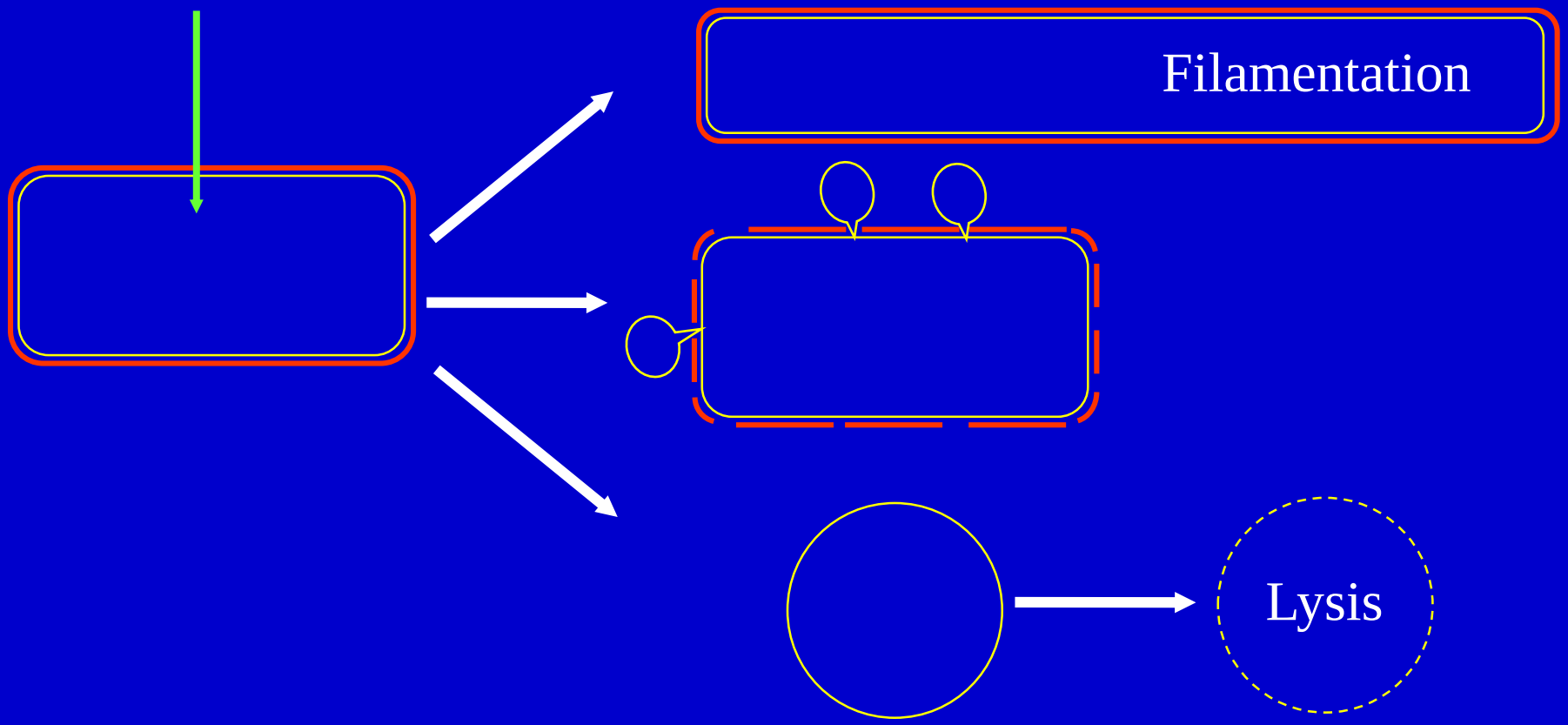
- **Inhibition of cell-wall synthesis**
  - inhibition of peptidoglycan cross-linking (beta-lactams)
  - inhibition of peptidoglycan synthesis (vancomycin)
- **Disruption of cell membrane**
  - polymyxins
- **Inhibition of protein synthesis**
  - at 30S ribosomal subunit (aminoglycosides, tetracyclines)
  - at 50S ribosomal subunit (macrolides, chloramphenicol)
- **Inhibition of nucleic acid**
  - inhibition of folic acid synthesis (sulphonamides, trimethoprim)
  - inhibition of DNA gyrase (fluoroquinolones)
  - inhibition of RNA synthesis (rifampin)

SELECTIVE TOXICITY !!!


Cell Wall Construction
Bacitracin
β-lactams
Fosfomycin
Glycopeptides
Structure and Function of the Cell Membrane
Colistin
Polymyxin B
Cell wall
Folic Acid Synthesis
Trimethoprim
Sulfonamides
THF
DHF
PABA
DNA
RNA
Protein
Cell membrane
PABA = para-aminobenzoicacid
DHF = Dihydrofolate
THF = Tetrahydrofolate
Structure and Function of DNA
Quinolones
Nitrofurantoin
Nitrimidazole
Rifampin
Protein Synthesis
Aminoglycosides
Lincosamide
Macrolides
Tetracyclines

I. Inhibition of cell wall synthesis (bactericid)

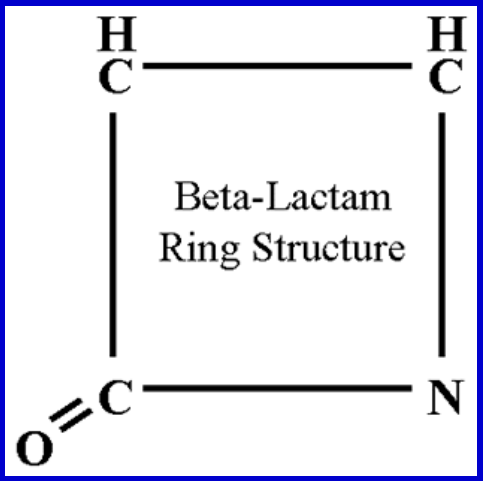
Cell wall controls osmotic pressure



I.1. β-lactams

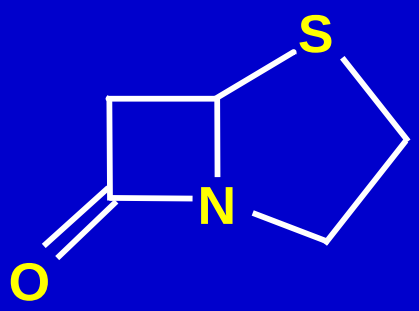
- **Inhibit transpeptidation of peptidoglycan chains**
- **Important questions:**
  - can be given orally? (acid stability)
  - β–lactamase (enzyme-) stability?
  - good against Gram negatives? (*Pseudomonas*, *Acinetobacter*!)

Structure of β–lactam ring:
(very vulnerable!)



I.1.1. Penicillins

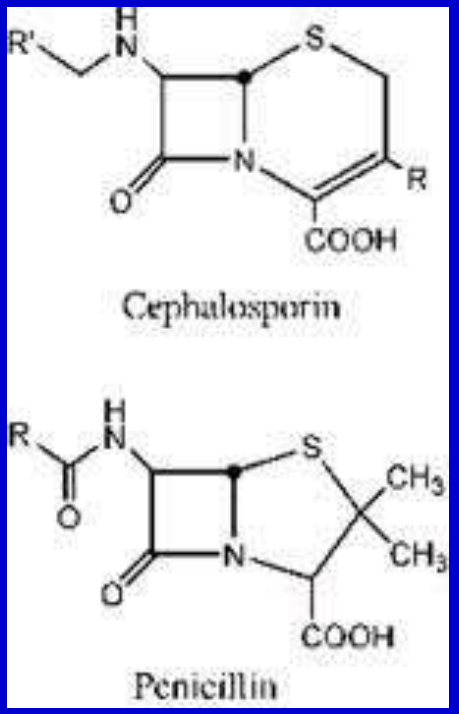
β–lactam ring
+ 5 membered /=tiazolidin-/ ring with sulphur



- natural penicillins: *penicillin G, V*
- enzyme stable: *methicillin*, *oxacillin* (**MRSA!!**)
- amino-penicillins: *ampicillin*, *amoxicillin* (given *per os*, but not enzyme stable)
- ureido-penicillins: *piperacillin*, *mezlocillin* (nor acid or enzyme stable, but good against *Pseudomonas*)
- carboxi-penicillins: *carbenicillin*

I.1.2. Cephalosporins

β–lactam + 6 membered /=cephem-/ ring with sulphur

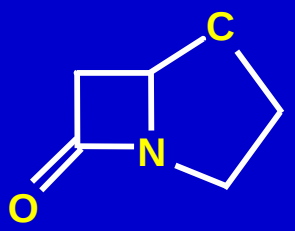


- more possibilities for substitution
- also against Gram negatives!

- I. gen.: *cefazolin, cephalexin, ...*
- II. gen: *cefuroxim, cefaclor, cefoxitin, ...*
- III. gen.: *cefotaxim, ceftriaxon, ...*
- IV. gen.: *cefepim, cefpirom*
- V. gen.: *ceftaroline, ceftobiprol*

I.1.3. Carbapenems

- widest spectrum!
- derived from penicillins
- *imipenem*, *meropenem*, *ertapenem*
- class B β–lactamase = carbapenemase

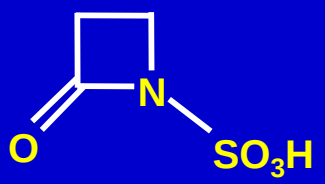


I.1.4. Carbacephems

- derived from cephalosporins
- *loracarbef*

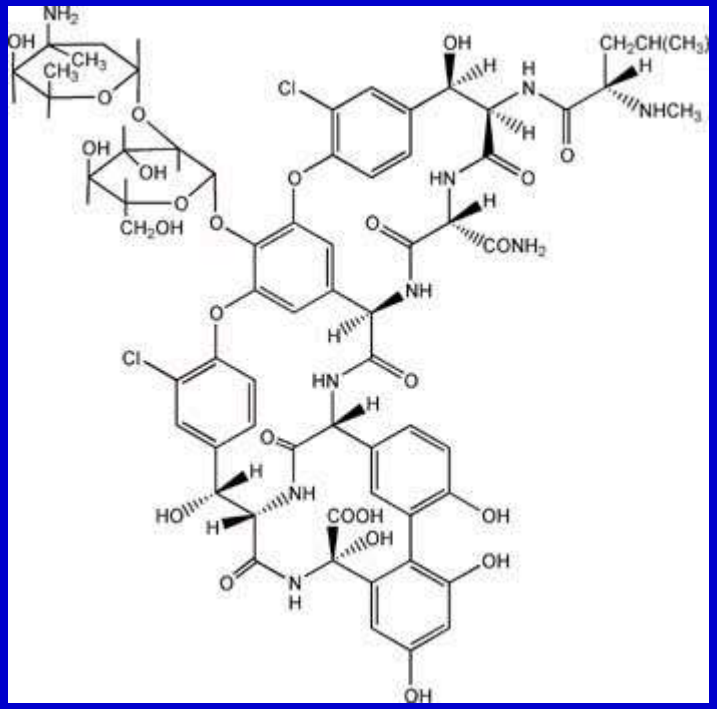
I.1.5. Monobactams

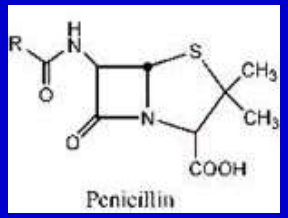
- *aztreonam*



I.2. Glycopeptides

- *vancomycin*, *teicoplanin*
- giant molecules
- triple effect:
  - cell wall synthesis
  - membrane permeability
  - DNA synthesis (?)
- last resort antibiotics
- **VRE!!**





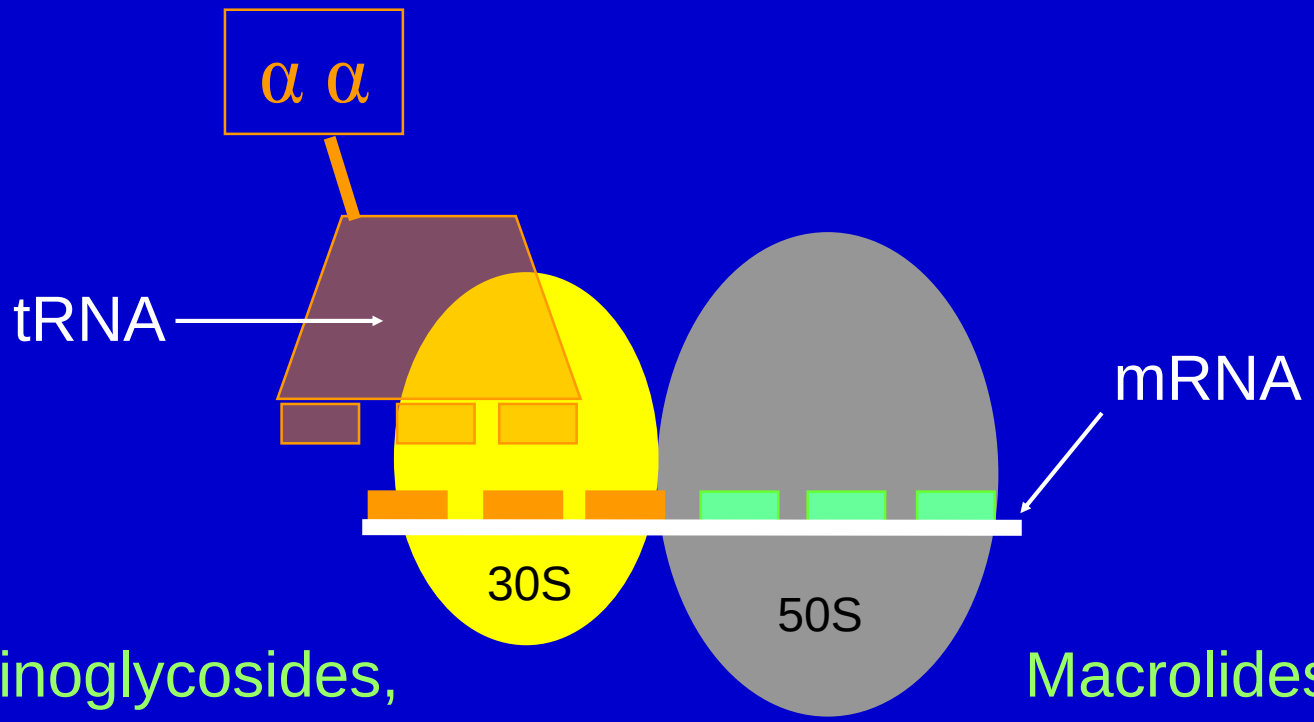
I.3. Polypeptides

- *Bacitracin*:
  - mainly against *S. aureus* and *Str. pyogenes*, for local treatment (skin infections)
  - by *Bacillus licheniformis*
  - inhibits cell wall synthesis

II. Disruption of cell membrane

- *Polymixins (e.g. Colistin)*:
  - desintegration of cell membrane
  - against Gram-negatives, for local treatment
  - (burns, ear, eye - *Pseudomonas*!)
  - bactericid, narrow spectrum

III. Inhibition of protein synthesis (usually bacteriostatic)



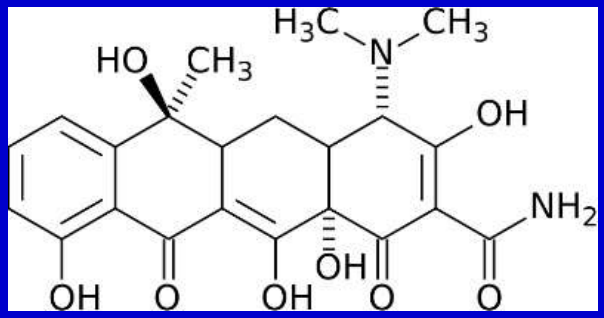
Aminoglycosides, tetracyclines

Macrolides, chloramphenicols

III.1. Aminoglycosides

- bactericid!
- act on 30S ribosomal subunit
- *streptomycin*: also against TB (today: only)
- today mainly:
  - *amikacin*, *netilmycin*: severe systemic infections
  - *tobramycin*, *gentamicin*: parenteral or eye drops
  - *neomycin*: eye drops
- **often toxic** (deafness!, kidney failure)

III.2. Tetracyclines



- *chlortetracyclin*, *doxycyclin*, *oxytetracyclin (Tetran)*
- act on 30S ribosomal subunit, inhibiting the binding of aminoacil-tRNA
- very wide spectrum (also for animals!)
- active against IC bacteria!!
  - *Chlamydia*, *Mycoplasma*, *Rickettsia*
- side effects:
  - liver failure (pregnancy!), kidney failure
  - acculmulation in bones (teeth of children!)
  - severe diarrhoea, mucosal inflammation

III.3. Chloramphenicol

- acts on 50S ribosomal subunit
- *Streptomyces venezuelae* (Ehrlich)
- wide spectrum → dysbacteriosis !!
- today mainly for:
  - typhus abdominalis, amp^R *Haem. influenzae*
- but: often in developing countries (cheap)
- *per os*, or eye drops / ointments (Chlorocid)
- toxic effects:
  - bone marrow malfunction
  - *„Grey baby syndrome”* in newborns

III.4. Macrolides

- act on 50S ribosomal subunit
- inhibit the elongation of peptide chain
- higher concentration: becomes bactericid
- groups:
  - 14 membered ring: *erythromycin*, *clarithromycin*
  - 15 membered ring : *azythromycin*
  - 16 membered ring : *josamycin*
- wide spectrum (*Streptococci*; *Bordetella*, STD, RTI /*Haemophilus*, *pneumo*/, *Helicobacter*, *Chlamydia*)
- cross resistance exists!

III.5. Lincosamides

- *clindamycin*, *lincomycin*

III.6. Streptogramins

- *quinupristin*, *dalfopristin*
- in combination = Synercid

III.7. Ketolids

- *telithromycin*

III.8. Oxazolidinons

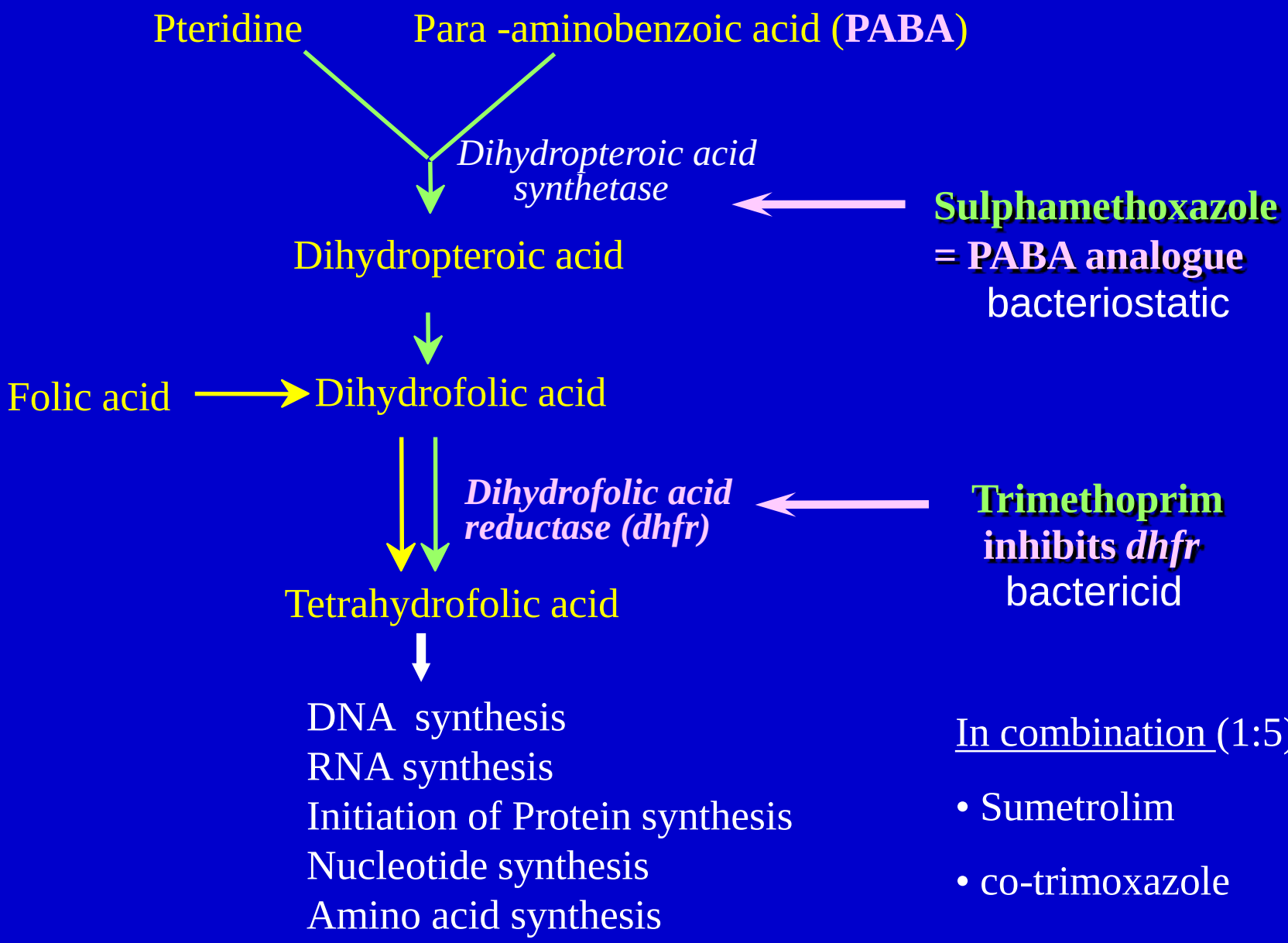
- *linezolid*

IV. Inhibition of nucleic acid synthesis

IV.1. Quinolons

- inhibition of DNA gyrase (supercoiling)
- original compound: nalidixic acid
- fluoroquinolones (FQs):
  - *ciprofloxacin*, *ofloxacin*, *norfloxacin*, *sparfloxacin*
- wide spectrum (also IC !)
- newer FQs (wider spectrum, better activity) – mainly against Gram-positive upper RTI:
  - *levofloxacin*, *moxifloxacin*, *gatifloxacin*, *gemifloxacin*
- Not in pregnancy or for young children!

IV.2. Inhibitors of folate synthesis

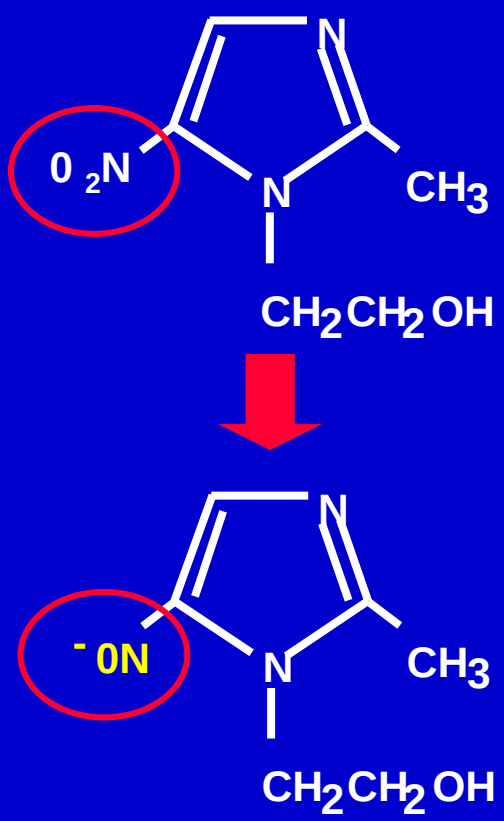


In combination (1:5):

- Sumetrolim
- co-trimoxazole

IV.3. Metronidazol

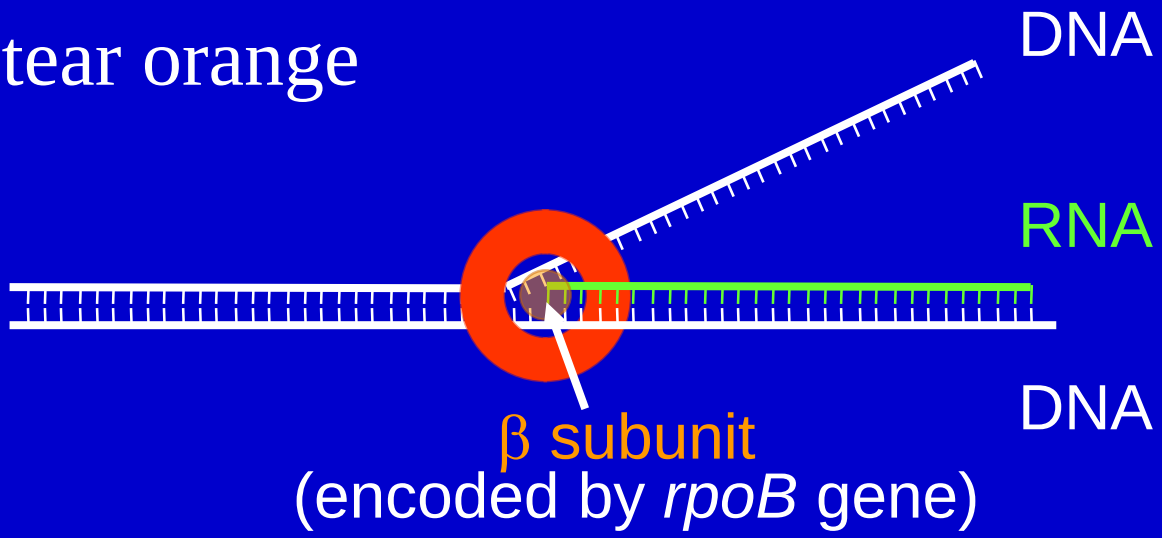
- against anaerobes + some protozoa
- directly breaks down DNA



- activated in the host cells by reduction of the nitro group at low redox potential (anaerobes!)

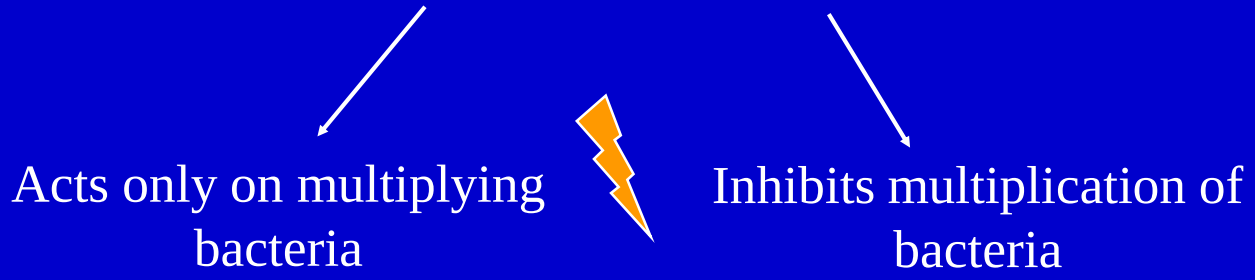
IV.4. RNA synthesis inhibition
Rifampin

- inhibition of DNA dependent RNA polymerase by binding to its β subunit
- if polymerisation has started already, it is ineffective
- paints tear orange



Aim of combinations

- synergy
  - Sumetrolim: TMP + SMX
  - Synercid: quinupristin + dalfopristin
  - penicillin + gentamycin
- avoiding resistance
  - ß-lactam + enzyme inhibitors
- polymicrobial infection
- **contraindicated**:
  - ß-lactam + bacteriostatic !!



RESISTANCE TO ANTIBIOTICS

First emergence of resistance

- 1928: discovery of penicillin
- 1940: first identification of a β-lactamase
- 1945: 50% resistance to penicillin in *Staphylococcus aureus*

Antibiotic resistant *Mycobacterium tuberculosis*

•1943: discovery of streptomycin

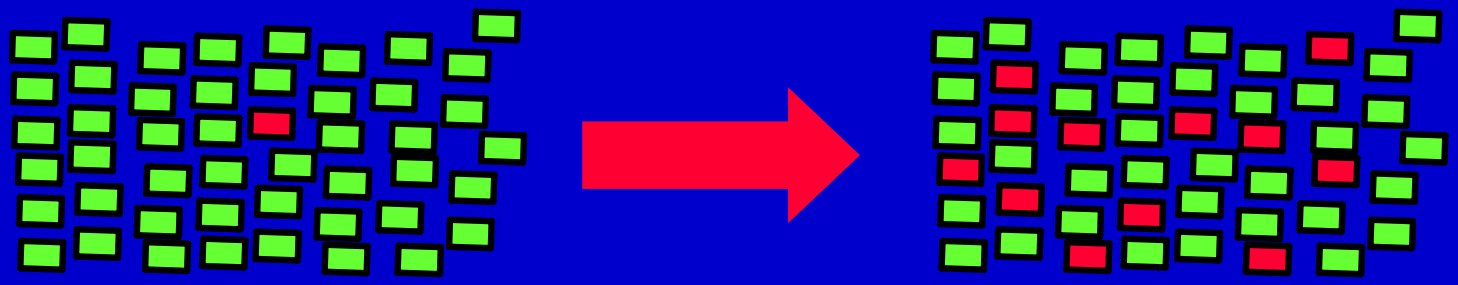
• 21 January 1950: George Orwell died from an untreatable streptomycin-resistant strain of *Mycobacterium tuberculosis*

Natural resistance

- against the antibiotic produced by themselves
- cell wall barrier (Gram-negatives), or lack of cell wall (*Mycoplasma*)
- lack of transport system
- lack of receptors

Acquired resistance - 1

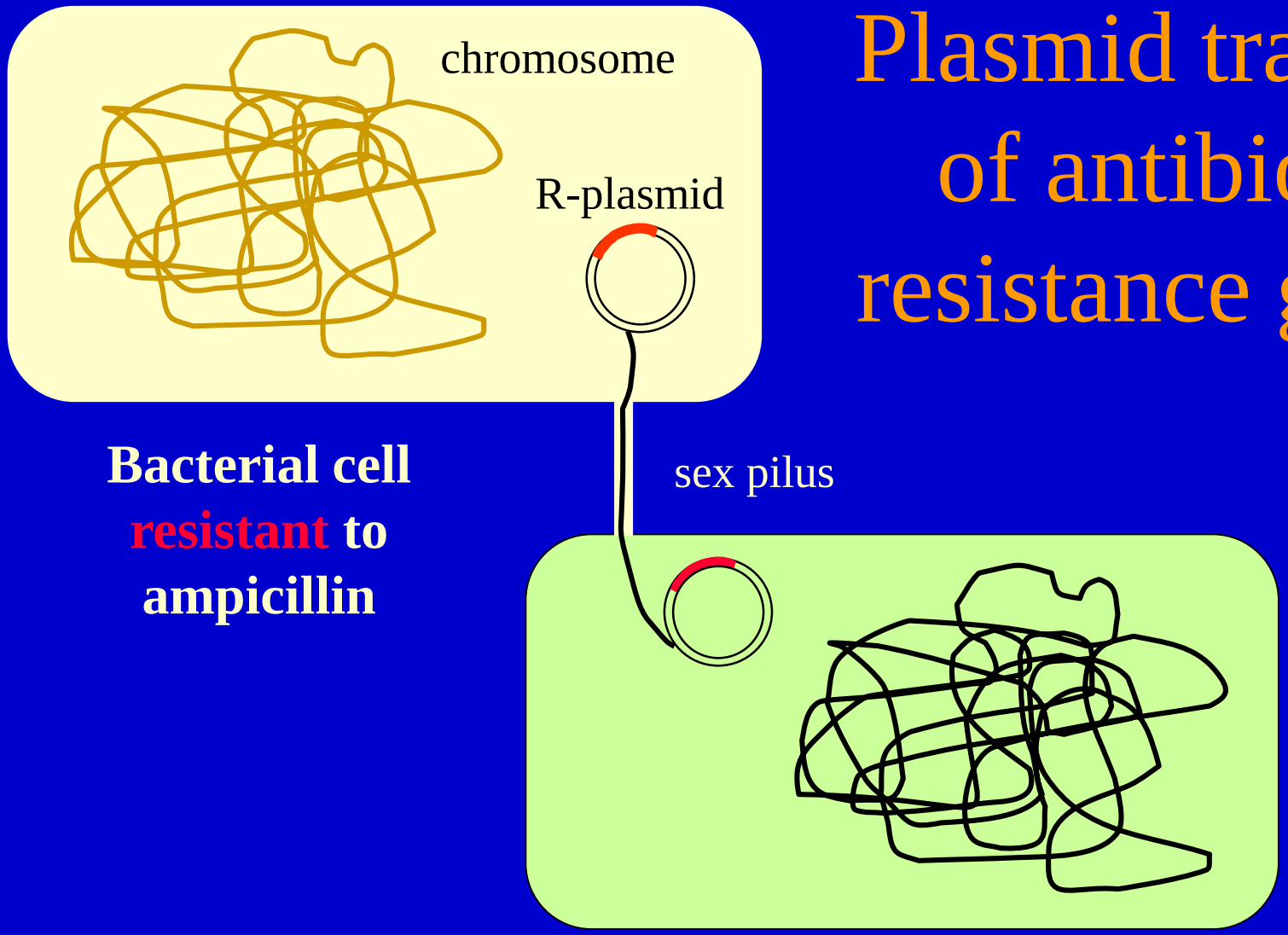
- **vertical**: spontaneous mutations (evolution, selection)
- normal mutation rate: 1 in 10^7
- selection of resistant mutants:



Acquired resistance - 2

- **horizontal**: giving resistance genes to other bacteria
  - by plasmid (conjugation)
  - by phage (transduction)
  - by transposon (mobile genetic elements)
  - by transformation (naked DNS)

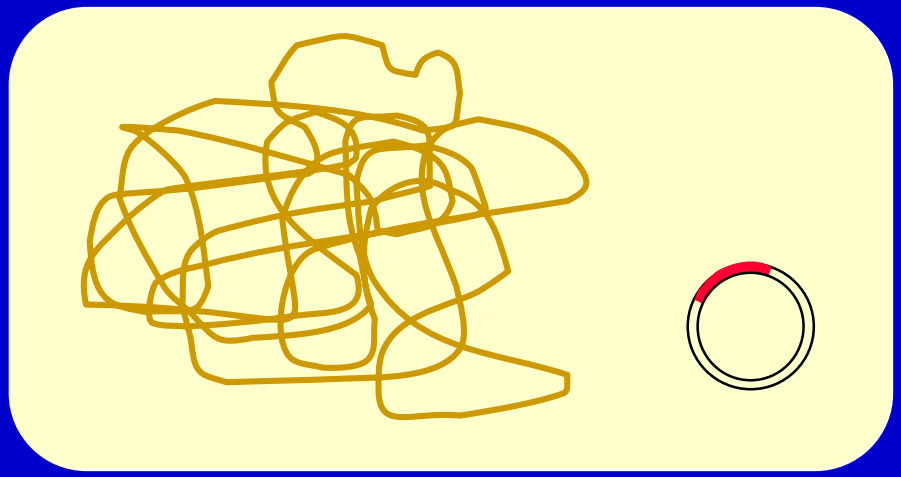
Plasmid transfer of antibiotic resistance genes



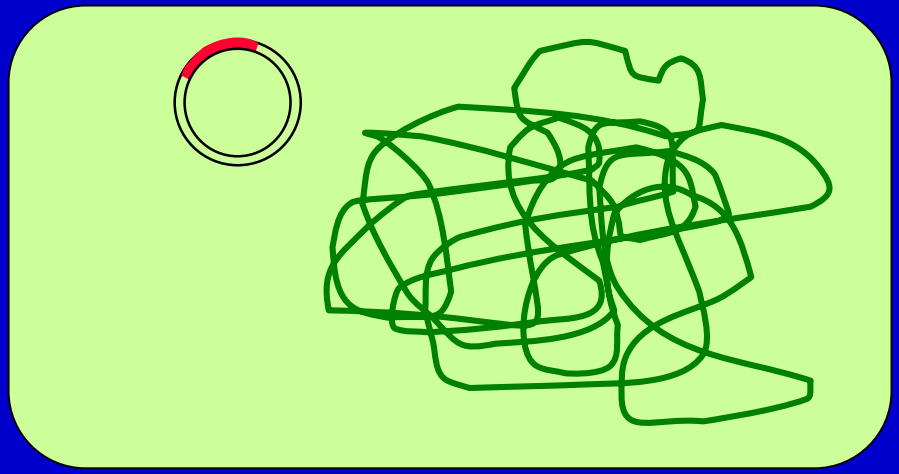
Bacterial cell resistant to ampicillin

Bacterial cell sensitive to ampicillin

Plasmid transfer of antibiotic resistance genes



Bacterial cell resistant to ampicillin



Bacterial cell RESISTANT to ampicillin

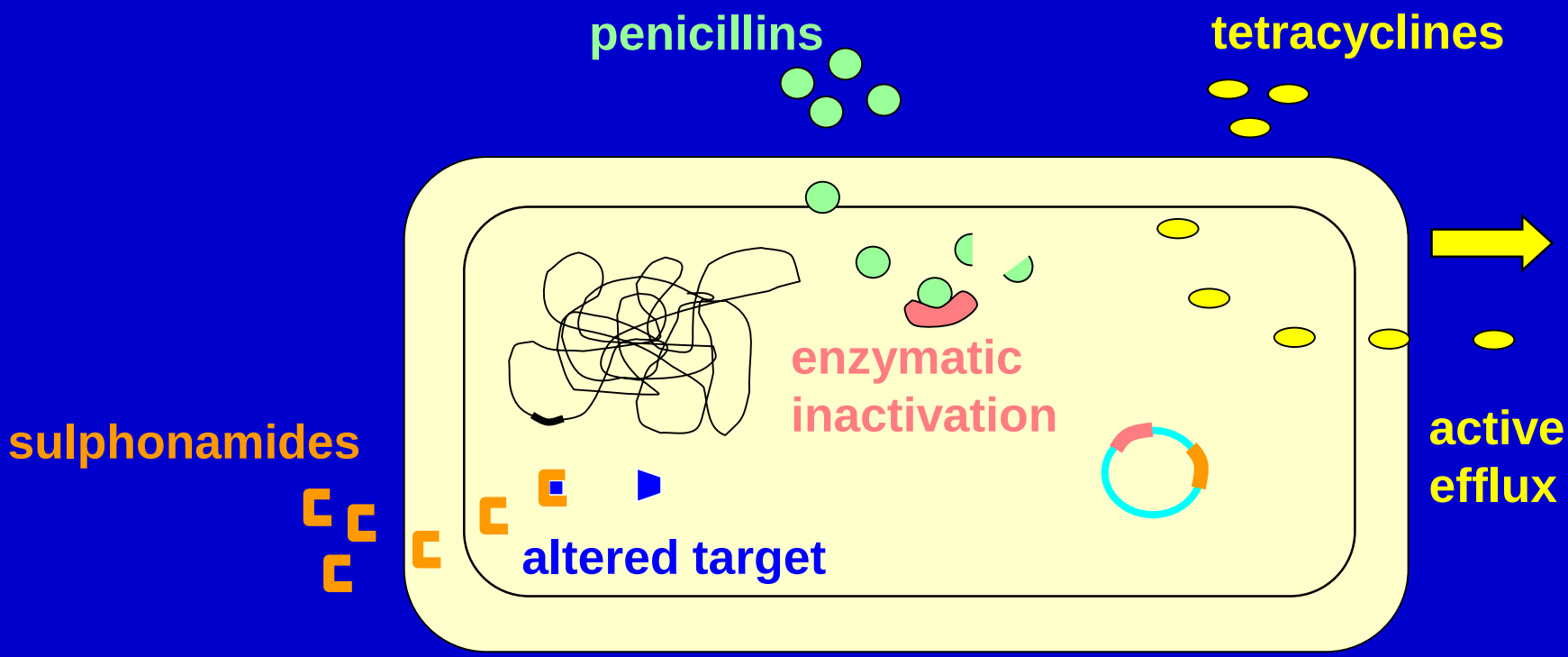
Human reasons leading to resistance

- prescribing antibiotics too often
- too long therapy, too low dose
- stop taking the antibiotic before completing the therapy
- usage of antibiotics in animal husbandry
- spread of resistant hospital strains (hygiene!)

MULTI DRUG RESISTANCE !!!

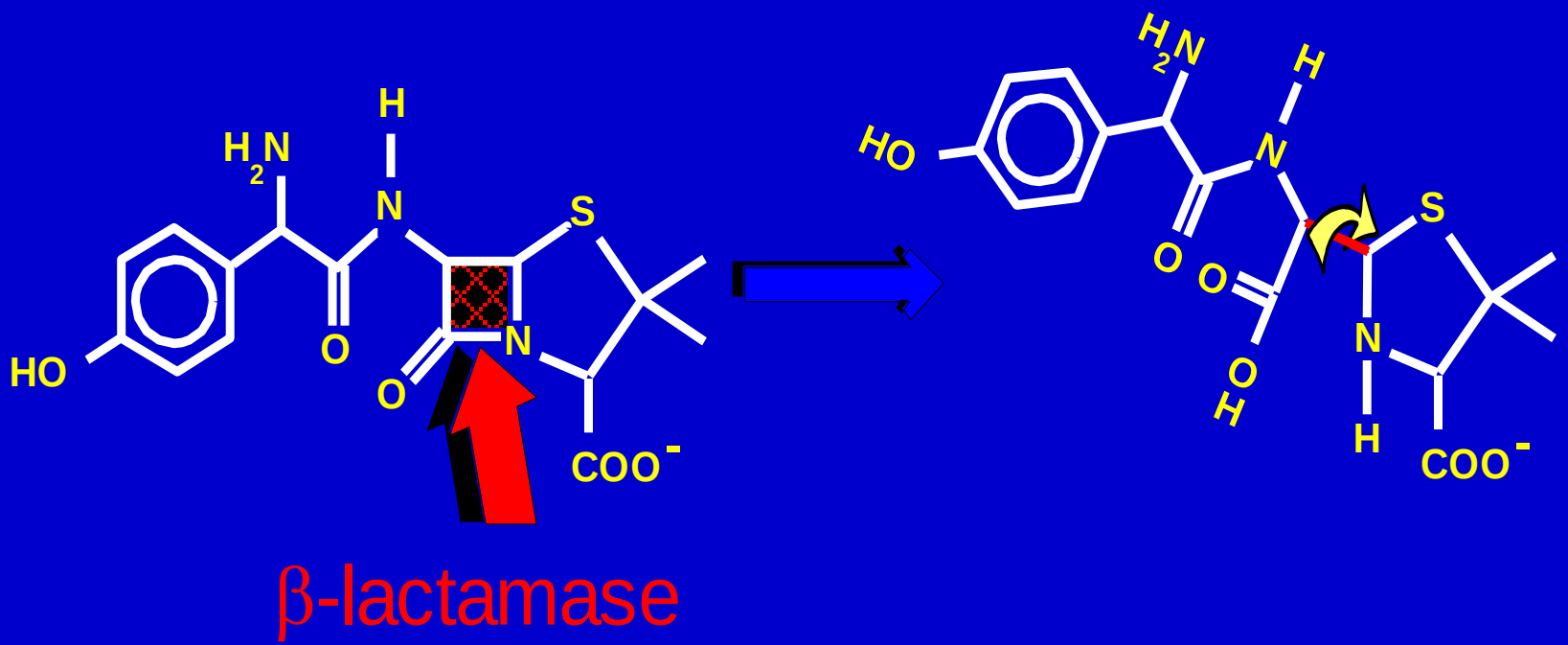
RESISTANCE MECHANISMS

The 3 major mechanisms



1. Enzymatic inactivation - 1

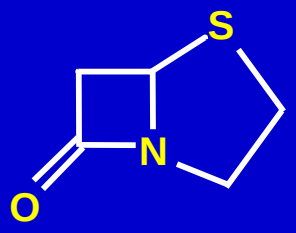
- cleaving (hydrolysis) of antibiotics !!
  - e.g. **β–lactamase** action on *ampicillin*:



Penicillin + enzyme inhibitor combination

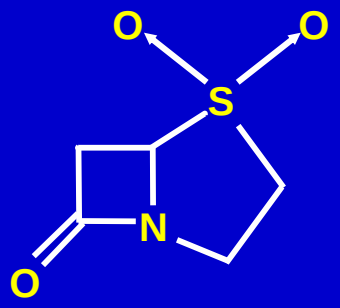
- enzyme inhibitor = β–lactam analogue (suicidal molecules)

- ampicillin-sulbactam = Unasyn
- amoxicillin-clavulanic acid = Augmentin
- piperacillin-tazobactam = Tazocin



penicillin

clavulanic acid



sulbactam

β-lactamases

- very many different ~
- mostly plasmid-encoded (sometimes chromosomal)
- constitutive or inducible (= in the presence of the β–lactam)
- **ESBL:** **extended spectrum β–lactamases !!**

  TEM, SHV, CTX, OXA

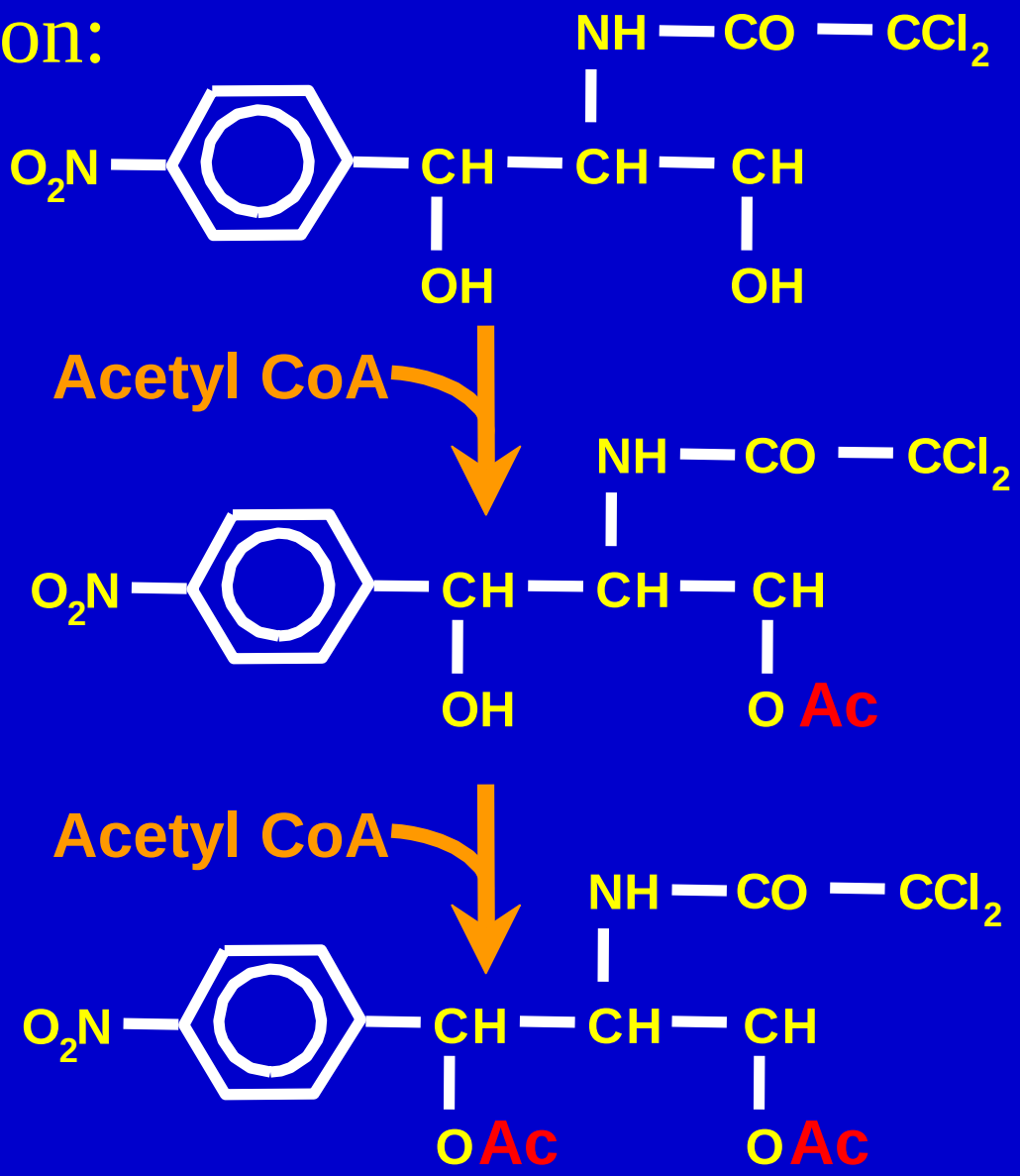
  by Gram negative bacteria

  (*E. coli*, Klebsiella, Pseudomonas, Acinetobacter, …)

1. Enzymatic inactivation - 2

- chemical modification:
  - acetylation
  - adenylation
  - phosphorylation
  - methylation
- *aminoglycosides, chloramphenicol*

e.g. acetylation of chloramphenicol:



2. Alteration of target by mutation

- decreased or no affinity

- *penicillins (pbp),*
- *aminoglycosides and macrolides (30S and 50S ribosomal subunits),*
- *quinolons (gyrase genes: gyrA,B)*

3. Efflux pump

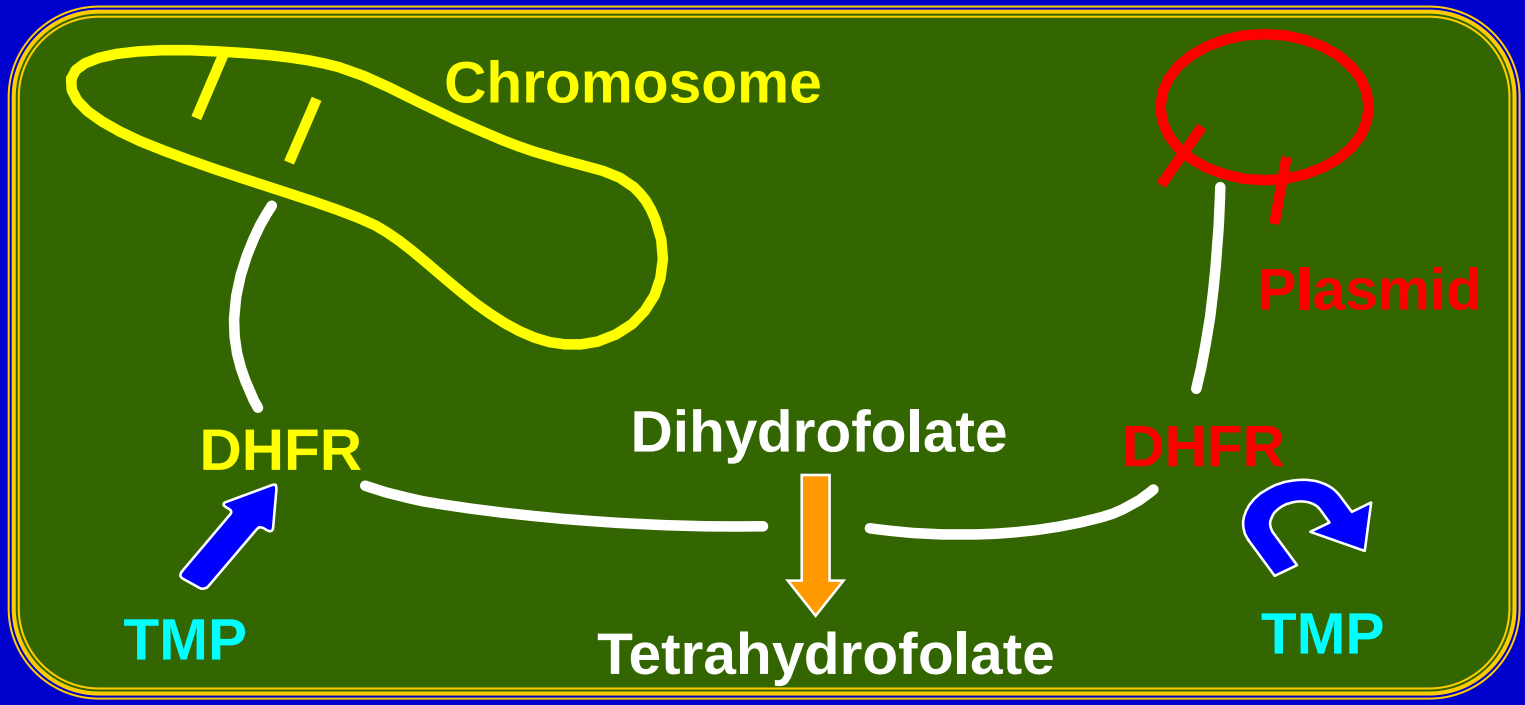
- removal of antibiotic
- not very effective
- *macrolides, quinolons, tetracycline*

4. Overproduction of targets

- e.g. overproduction of PABA *(SMX)*

5. Metabolite by-pass

- production of another target
  - e.g. an additional dihydrofolate reductase



6. Change of membrane permeability

- blocking active transport
- e.g. MRSA: altered membrane lipid structure
- *e.g. tetracycline*

7. Decreased modification to active component

- e.g. loss of nitrofurantoin-reductase

Problem bacteria

- *Staphylococcus aureus* – **MRSA, VRSA**
  (methicillin- and vancomycin resistance)
- *Enterococcus faecalis* and *faecium* – **VRE**
  (vancomycin resistance)
- MDR, XDR *Mycobacterium tuberculosis*
- Carbapenem resistant Gram negatives
  - *Acinetobacter baumannii*
  - *Pseudomonas aeruginosa*
  - *Klebsiella* spp.
  - *Stenotrophomonas maltophilia*

ESBL

DETERMINATION OF ANTIBIOTIC SENSITIVITY

Disc diffusion test

- Based on zone diameter:
  - **R** (resistant)
  - **I** (intermediate)
  - **S** (sensitive)

- this is used in routine
- good for screening

“antibiogram”

Determination of MIC

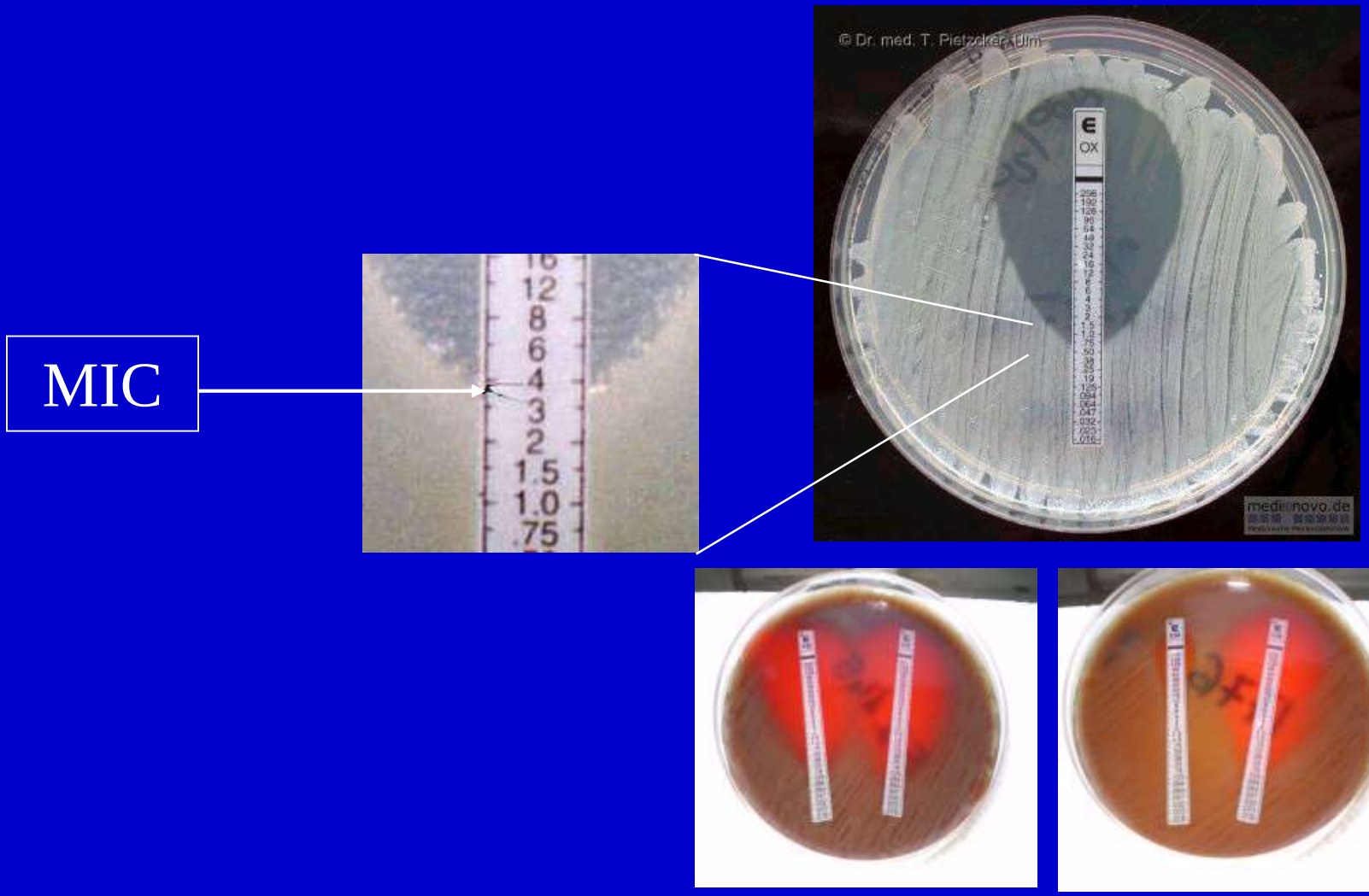
- definitions:
  - **MIC = minimal inhibitory concentration**

    = the minimum concentration (in mg/L) of an antibiotic enough to inhibit the growth of a certain bacterial isolate

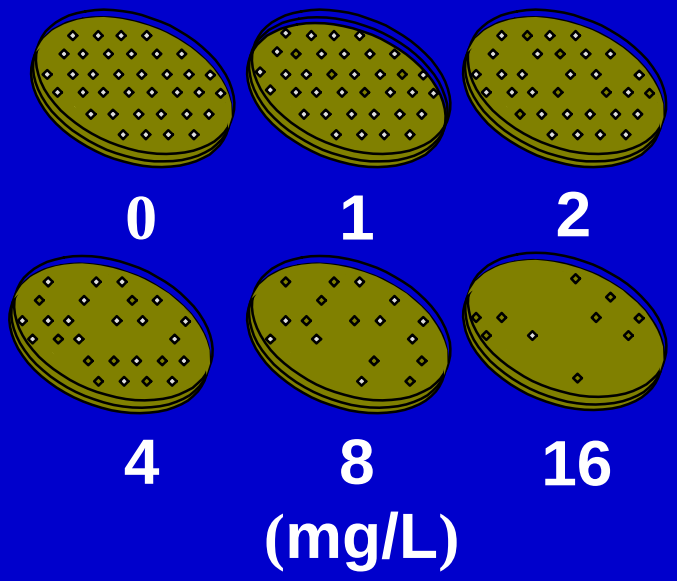
  - MBC = minimal bactericid concentration

MIC determination by diffusion

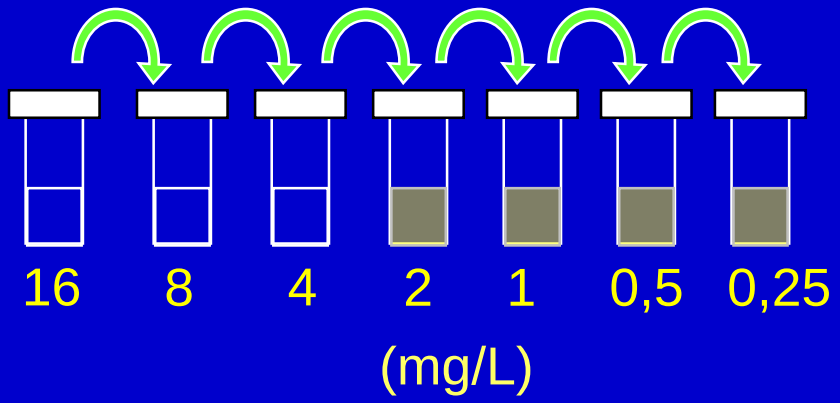
- **Etest**: concentration-gradient on a strip



MIC determination by dilution



agar dilution (AB mixed into the medium)



broth dilution (AB mixed into the medium)