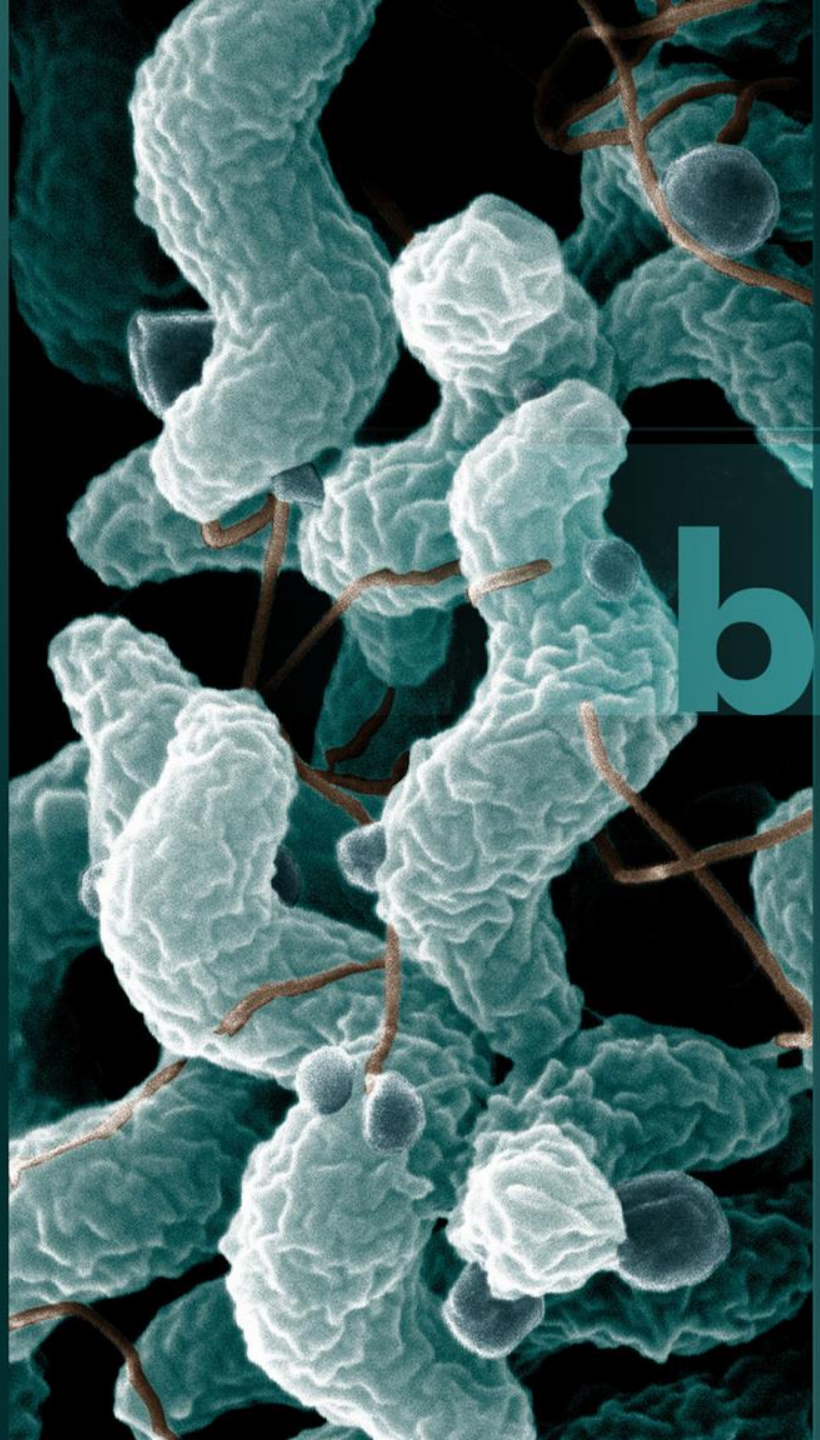
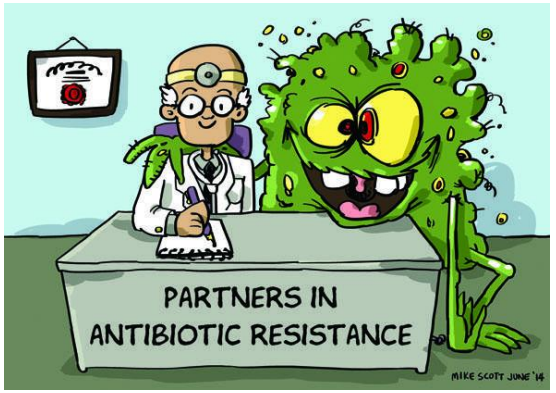


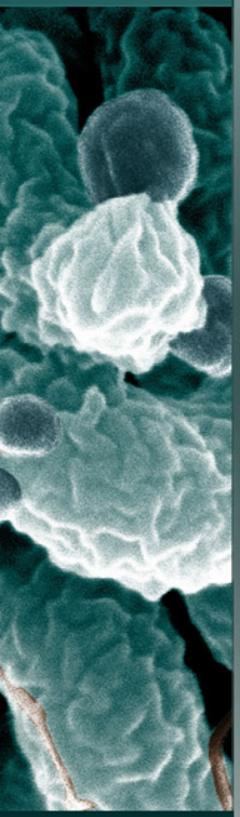
6th Seminary of microbiology
FaF VFU BRNO

bacteria

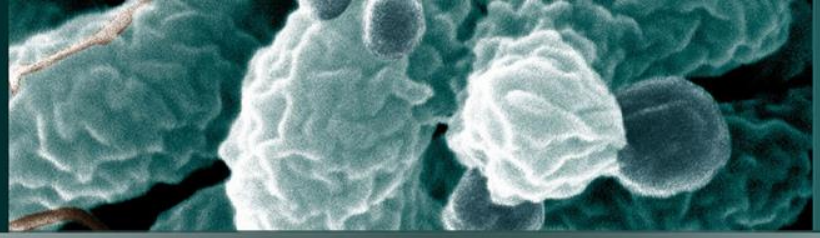
Bacterial resistance
Synergy
Biofilm

Bacterial resistance





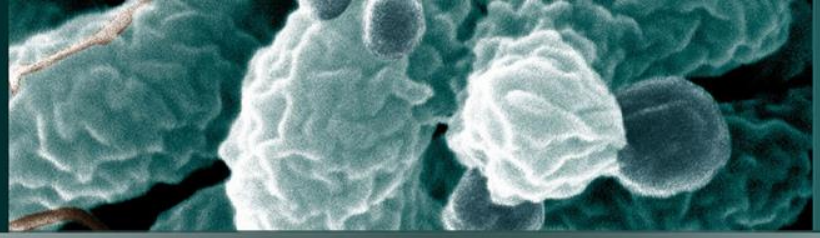
- Bacterial infections were, are and will be one of the most serius problems in medicine.
- Usually endogenic character – arise from therapeutic and diagnostic procedure.
- Infections caused by resistant strains in hospital patients have **higher mortality** compared to infections caused by sensitive strains of the same bacteria (↑ costs – more expensive ATB, paatient isolation and prolonged hospital stay).



Bacterial resistance

- **Resistance to an ATB** is ability of bacterial population **survive the effect of inhibiting concentration** of given antimicrobial product.

- Resistant strain contains genes of resistance to one or more ATBs.
  - **natural** or primary
  - **acquired** or secondary



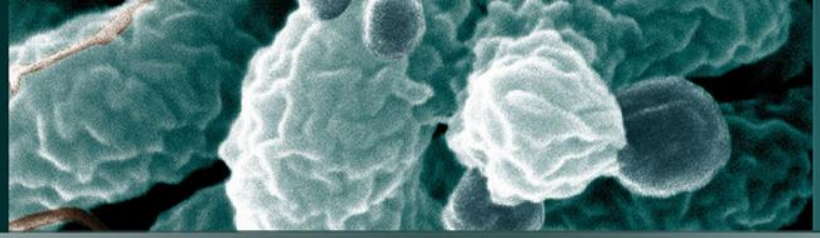
Natural resistance

- **Innate insensitivity** of certain bacterial species to an ATB.
  - Lack of target for the ATB
  - Inactivation of the ATB

- Typical for all members of that species
- Coded by chromosomal genes
- Horizontal gene transport impossible.

- Applies in evolution of microbes as an mechanism of adaptation to new conditions in environment.

- *Beta-lactamases probably evolved from enzymes responsible for synthesis of peptidoglycans (PBP).*



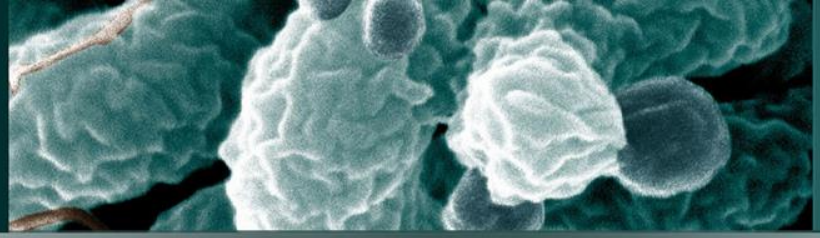
Acquired resistance

- Secondary resistance can arise by two means:
  - phenotypic adaptation
  - genetic changes

- Conjugation – sex pilus – transfer of plasmids, transposones
- Transformation – absorption of free NA from outside
- Transduction – genetic information is part of phage NA - bacteriophages

Factors:

Selection pressure and overusage of ATBs in human and veterinary medicine and agriculture.



Key concepts

MULTIRESISTANCE

= resistance of microorganism to more than one ATBs

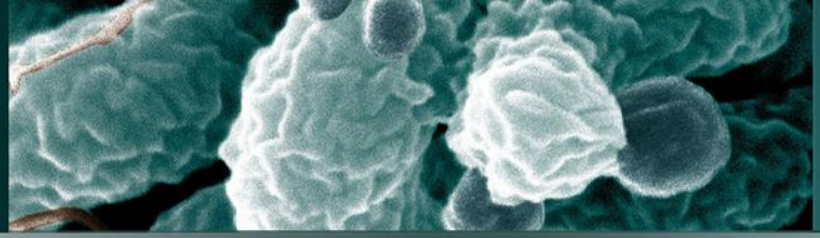
- Methicilin-resistant *Staphylococcus aureus* (MRSA)
- Vancomycin-resistant enterococci (VRE)
- *Enterobacteriaceae* producing broadspectrum beta-lactamase (ESBL) (*E. coli* a *K. pneumoniae*)
- Multiresistant *Pseudomonas aeruginosa*
- *Clostridium difficile*

CROSS RESISTANCE

= to antibiotics, which act with same mechanism (penicilins and cephalosporins)

ASSOCIATED RESISTANCE

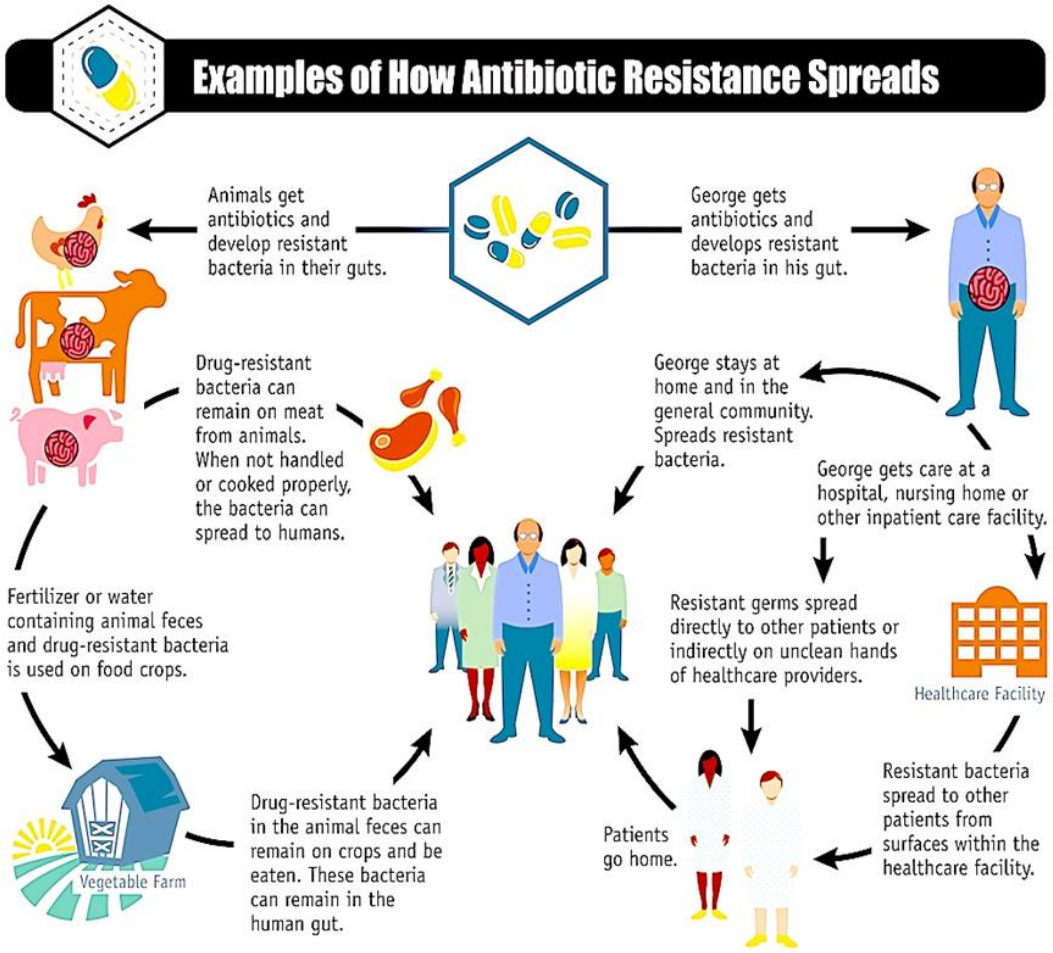
= genes are in clustres



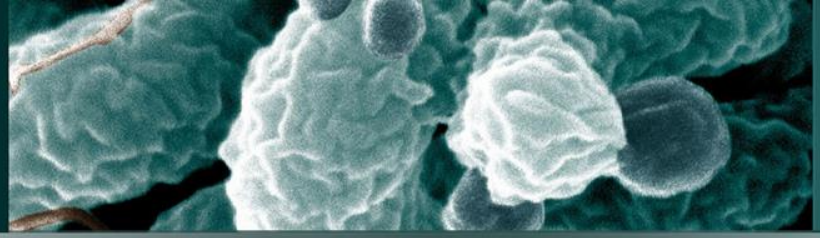
Spreading

Resistant strains are in:

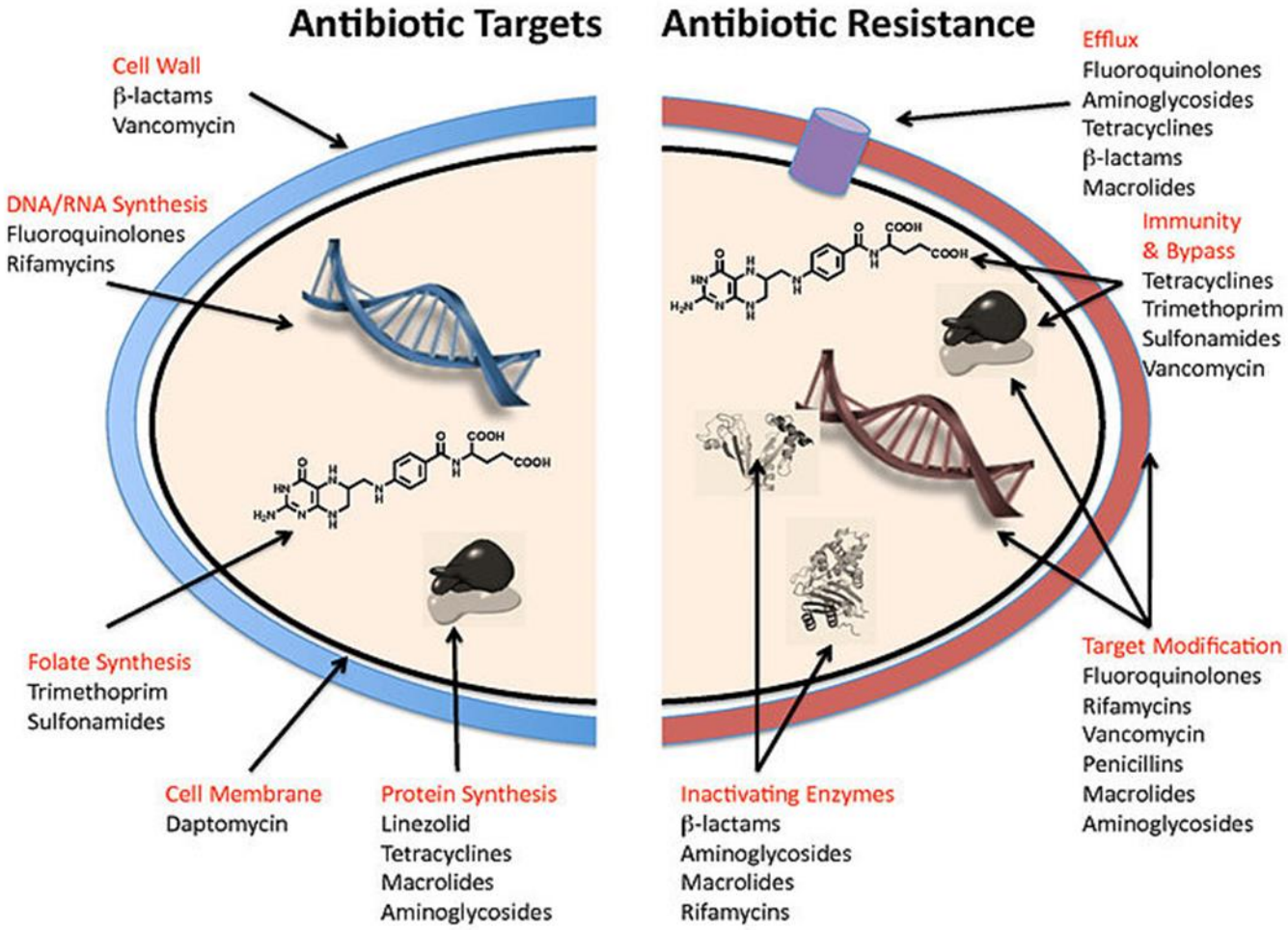
- People
- Animals (big farms)
- Foodstuff
- Environment (air, water, soil)
- Hospitals



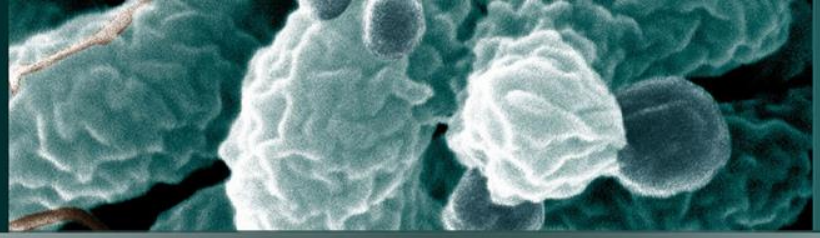
http://www.care2.com/greenliving/antibiotic-drug-resistant-threat-growing-at-alarming-rate-warns-cdc-infographic.html



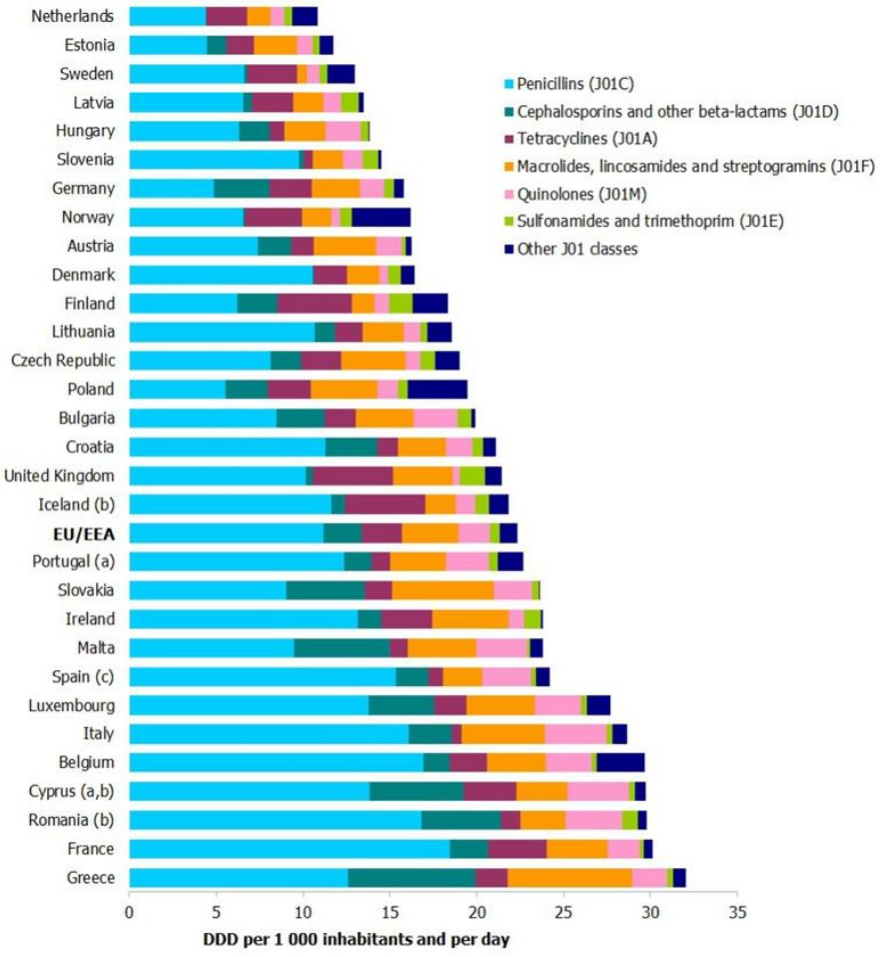
Mechanisms



https://en.wikipedia.org/wiki/Antimicrobial_resistance#/media/File:Antibiotic_resistance_mechanisms.jpg

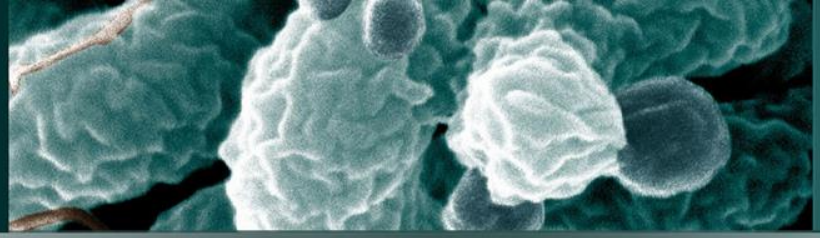


Resistant bacterias in EU

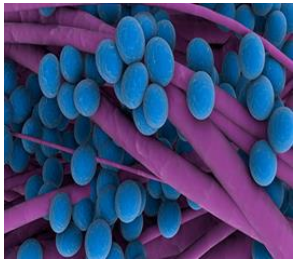


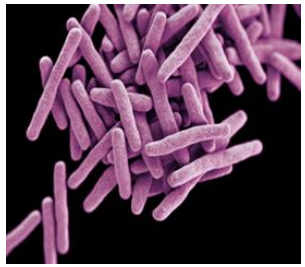
- 25 000 patients lose life p.a.
- 50% indications of ATB are needless
- 1,5 bilions € p.a. for complications in treatment
- max. 30% of infections are bacterial
- 80% of antibiotics are prescribed out of hospitals

http://www.zdravotnickydenik.cz/2015/11/rezistentnich-bakterii-v-eu-stale-pribyva-o-zivot-rocne-pripravi-25-tisic-lidi/



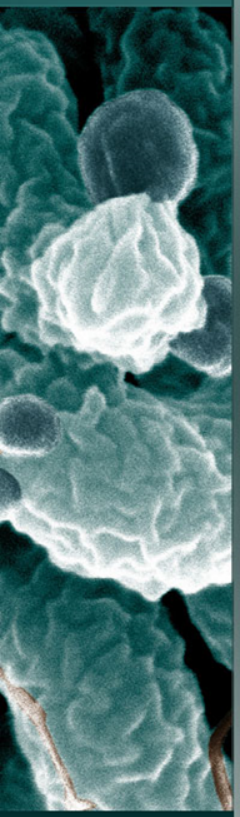
Clinically important



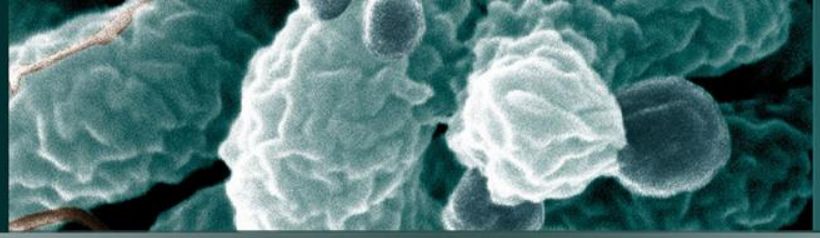


- Methicillin resistant *Staphylococcus aureus* (PEN, MET, TET, ERY, VAN, LIN)
- Multi-resistant *Mycobacterium tuberculosis* (ISO, RIF)
- Multi-resistant *Plasmodium falciparum* (ARTE)
- Multi-resistent *Neisseria gonorrhoeae* (CEF, AZI, TET, AMG)
- Multi-resistant *Acinetobacter*
- Multi-resistant *Pseudomonas aeruginosa*
- Carbapenem resistant *Enterobacteriaceae*
- Carbapenem resistant *Klebsiella*

Disinfectants have significant contribution to production of resistance



- in healthcare and agriculture and in households – everyday usage of disinfictants
- damaging of commonly present bacterias in environment
- effectivness is reduced by dilution
- it stimulates eflux pumps present in multi-resistant strains
- usage of disinfectants in community stimulated presence and spreading of MRSA.

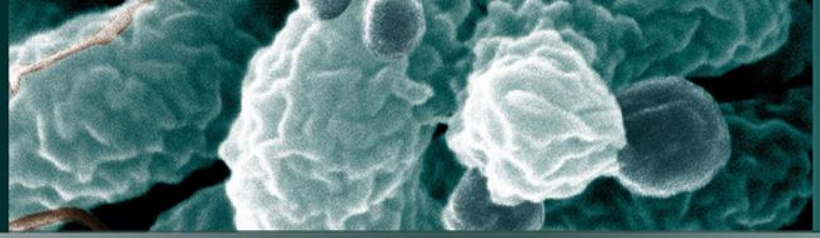


Clonal spreading

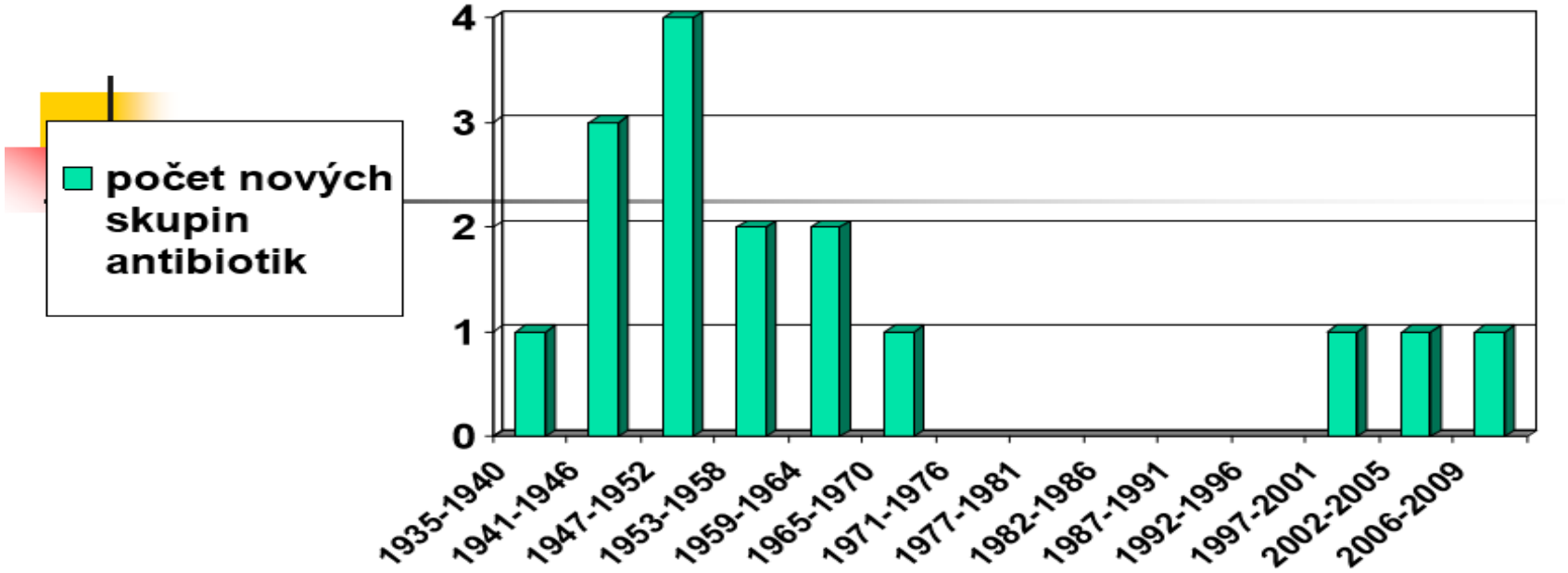
If there is acquisition of gene for resistance in a strain – it has higher abaility to survive and spread itself by clonal mulitiplication and spreading.

- faster colonisation of GIT
- survive more successfully
- can be mor pathogenic
- spread to environment – waste water, food comtamination

- *E. coli* producing broadspectrum beta-lactamase (ESBL) CTX-M-15.
- Clones of *K. pneumoniae* (ESBL, carbapenemase) belonging to clonal complex (CC) 11
- MRSA USA300

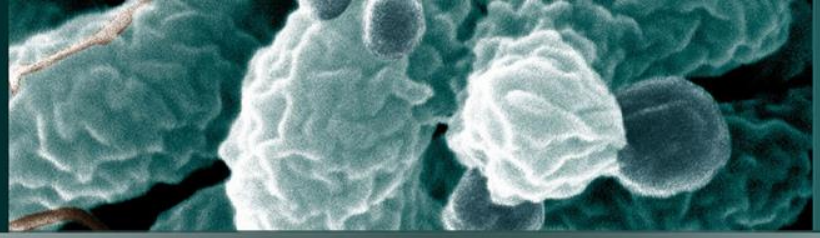


New ATBs



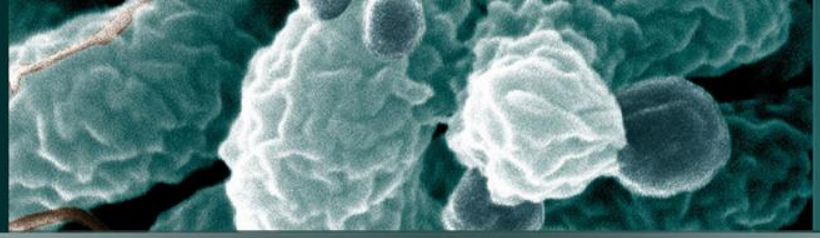
1935-1940: sulfonamidy
1941-1946: peniciliny,aminoglykosidy,cefalosporiny
1947-1952: chloramfenikol,tetracykliny,
makrolidy,linkosamidy
1953-1958: glykopeptidy,rifamyciny
1959-1964: nitroimidazoly,chinolony
1965-1970: trimetoprim
1997-2001: oxazolidinony
2002-2005: lipopeptidy
2006-2009: glycylcykliny

http://is.muni.cz/el/1411/jaro2014/VSAT081/um/1__INT_Uvod_do_antimikrobialni_terapie__25.2.2014_.pdf



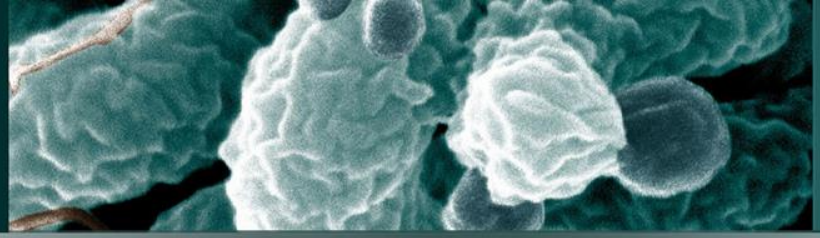
Irrational ATB therapy

- ATBs are safe drugs and it is always better to prescribe them
- application in case of potential pathogen presence or antibody titer in blood, without any other clinical or lab markers
- application of ATBs is always risky in sense of resistance
- ATB therapy should be aimed to clinically proven baterial infections



Common mistakes in ATB therapy

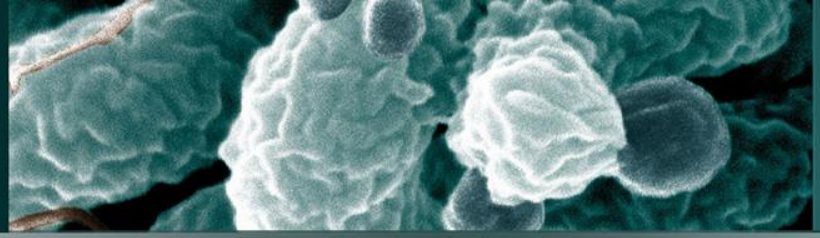
- ATB in therapy of non-infectious disease (should be antipyretic)
- prescription of ATB for banal respiratory disease (rhinitis - viral)
- subdosing of ATB (production of resistant clones)
- too long or too short therapy



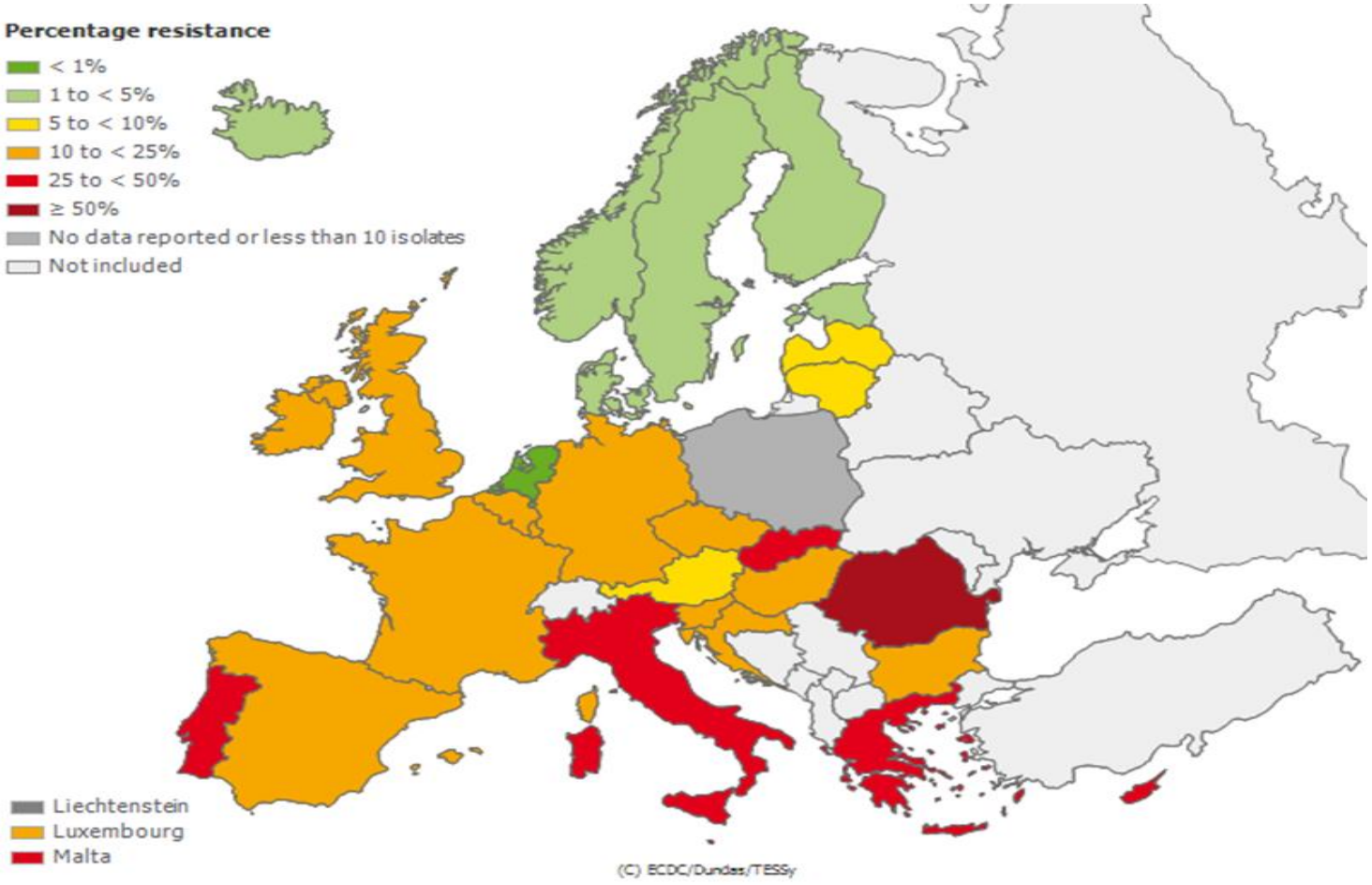
MRSA

Methicillin resistant *S. aureus*

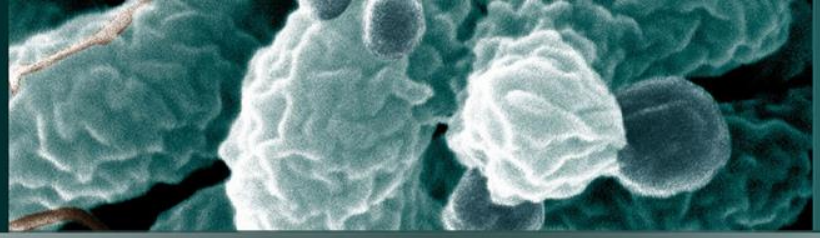
- CA-MRSA x HA-MRSA x LA-MRSA
- frequent post-traumatic/operative infection
- 24 – 48 hrs topical symptoms → tissues
- CA-MRSA: ↑ virulence caused by PVL, PSM, TSST-1, sepsis
- resistance to beta-lactam antibiotics (penicillins, cephalosporins)
- *mecA* (SCCmec): penicillin-binding protein 2a (PBP2a)- alternative transpeptidase
- other genes of resistance: tetracyclin, streptomycin, chloramphenicol, …



MRSA in EU



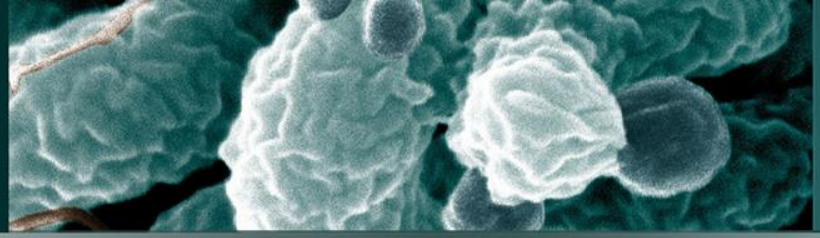
http://ecdc.europa.eu/en/healthtopics/antimicrobial_resistance/database/Pages/map_reports.aspx



Synergy „1+1>2“

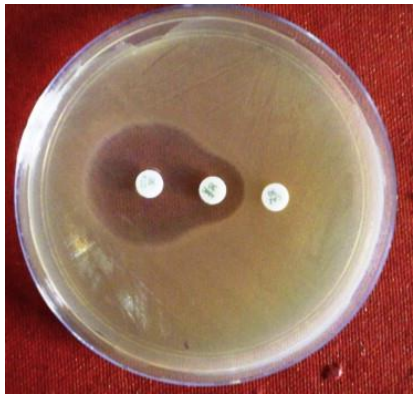
- multi-efector pharmacological effect
- influence on pharmacokinetics
- reduction of toxicity
- interaction with mechanisms of bacterial resistance:

1) deactivation of esterases (penicillinases)
2) interaction with PBPs
3) damage of peptidoglycan
4) inhibition of NA synthesis
5) blocking of eflux systems



Synergy „1+1>2“

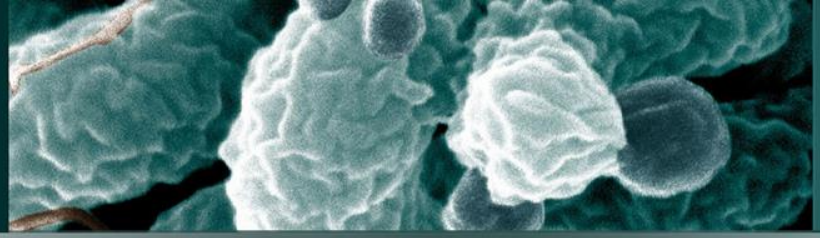
- 2 antibiotics
- antibiotic + potentiating agent (natural compound)



DEFINITION

= cooperation, joint action

- **final effect of compounds acting together is bigger than sum of effects of that compounds**



Combination therapy

Aditive effect:

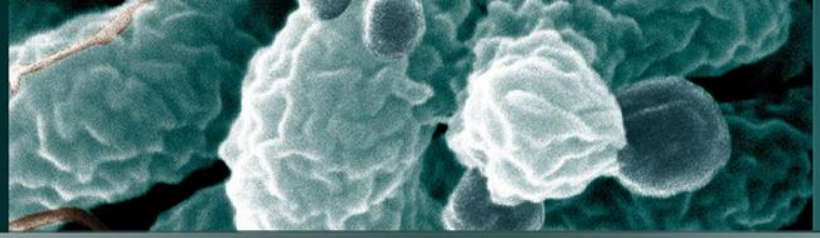
- effect of two compounds in combination is equal to sum of the individual effects

Synergic effect:

- effect of two compounds in combination is bigger than sum of the individual effects

Antagonistic effect:

- effect of two compounds in combination is smaller than sum of the individual effects



Indication for ATB in combinations

Prevention of resistance

- combination of β-lactam ATBs and aminoglycosids (to suppress production of β-lactamase)

Infections caused by more microorganisms

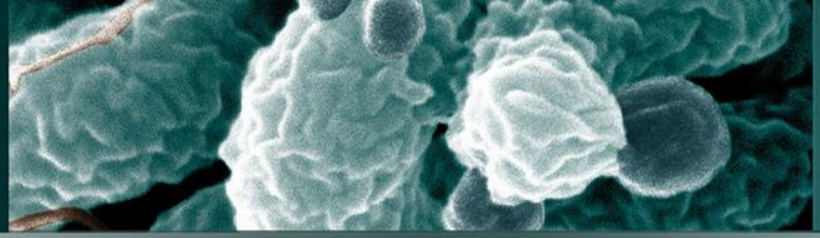
- abdominal infections: ciprofloxacin ane metronidazol
- mixed aerobic and anaerobic organisms

Initial therapy

- when the cause of infection is not known yet
- high doses of cephtriaxone with vancomycin in case of suspection of pneumococci meningitis in regions with high resistance to PEN

Reduction of toxicity

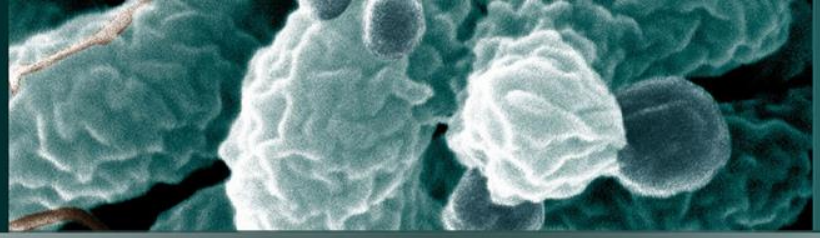
- reduction of the dose need to treat



Example of synergy

- **increased uptake of aminoglycoside ATB into bacteria in combination with β-lactam**

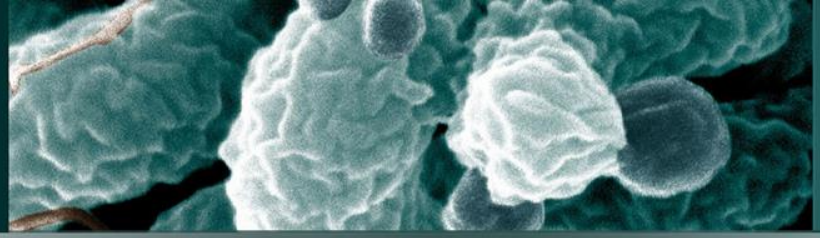
- treatment of enterococci endocarditis: ampicilin + gentamicin
- streptococci endokarditis: penicilin + gentamicin
- staphylococci bacteraemia: vancomycin + gentamicin
- pseudomonas infection: beta-lactam ATB + aminoglycoside



Wrong combinations

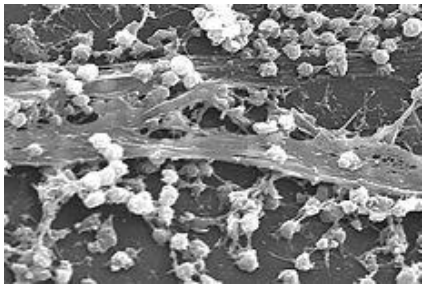
- **Antagonism (PEN+TTC)**
  - bactericidal compounds become bacteriostatic
  - serious for immunocompromized patients; isolated infections, where is inadequate reaction of host – meningitis, endocarditis
- **Higher costs**
- **Adverse effects (toxicity, allergic reactions)**
- **Superinfections (colonisation with resistantn strains)**
- **False feeling of safety**

- Chloramphenicol + erythromycin: in same infusion → precipitation, loss of activity
- Ticarcillin or carbenicilin with aminoglycosides → inactivation of aminoglycoside



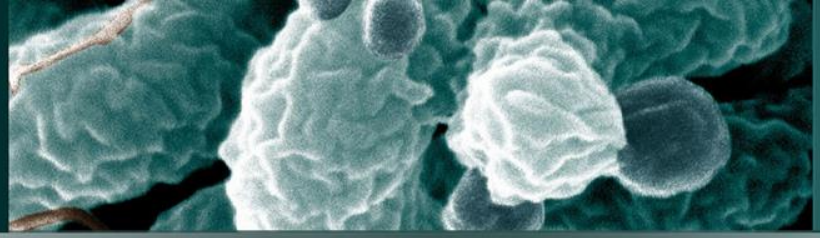
Biofilm

= microbial colony in extracellular matrix (polysaccharides), adhering to inert and living surfaces



- complex structure with channels (water brings nutrients and takes away waste)
- **similar to tissues** of higher organisms
- its formation frequently depends on more bacterial species

- bacteria are **shielded** in biofilm v biofilmu from environment and immunity system
- biofilm can be thick from several to hundreds mm

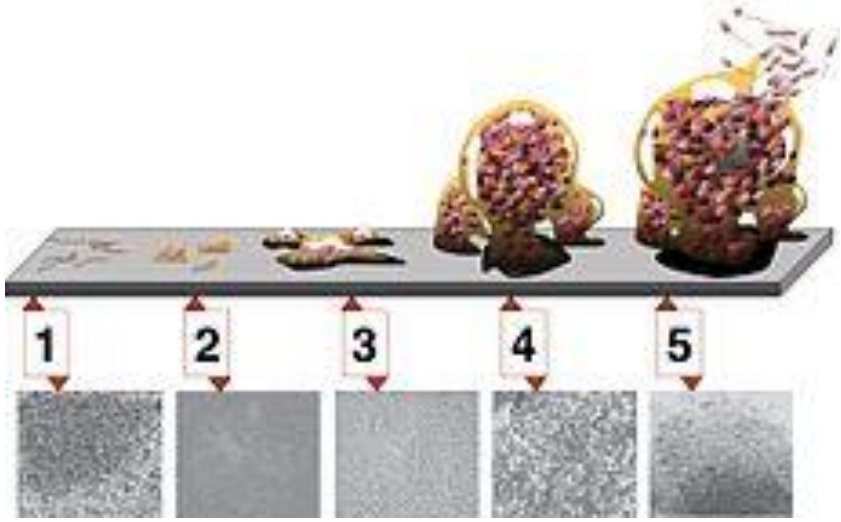


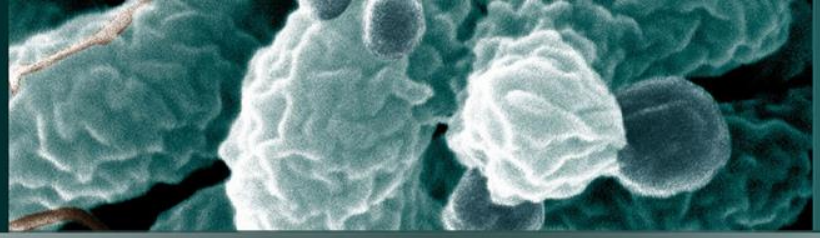
Biofilm

FORMATION

- adherence on surface → turning on genes for extracellular polymer formation → microcolonies with mucus → differentiation into biofilm → separation of bacteria and spreading
  - chemical signals turn on genes, active also in **quorum sensing** (activation of gene expression dependent on density of bac. population)

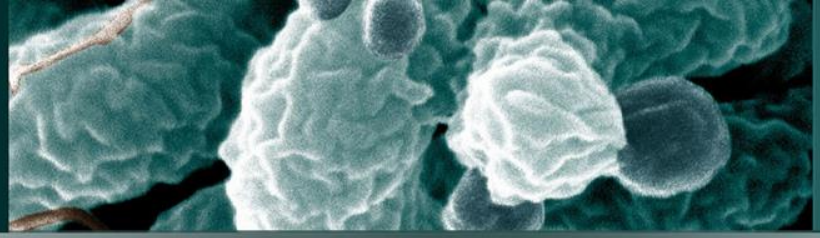
1. initial adherence
2. irreversibile adherence
3. maturation I
4. maturation II
5. separation and spreading





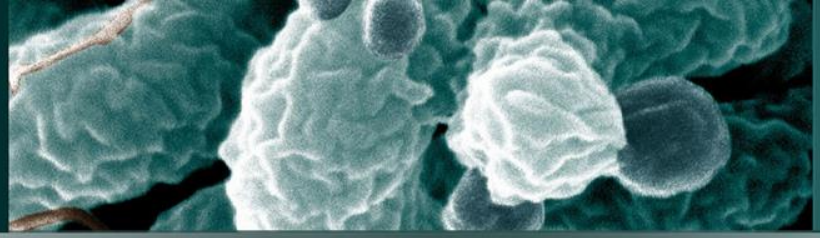
Resistance of bac. In biofilm to ATB

- cells in biofilm are very resistant to ATB and disinfection (1000x more)
- even high doses of ATB are not enough to treat it – lab results of sensitivity to ATBs can show wrong results
- resistance is expressed phenotypically – not genetic resisitance
- resistant cells, which tolerate ATBs and stay in the body are called persisters
- between the cells genes transfers even 1000x more efficiently than between planctonic (free) cells
- cells in biofilm are also protected from antibodies



Places where biofilm is formed

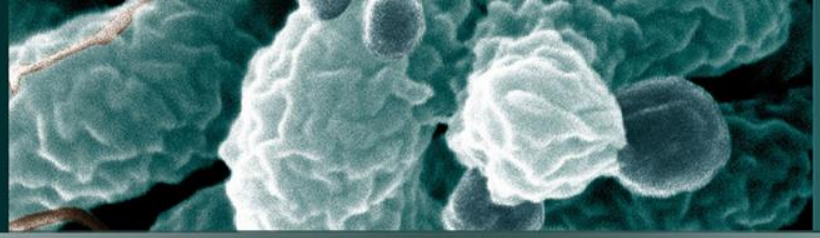
- **dental caries** (viridans streptococci)
- **periodontitis** – in pockets under gum
- **otitis media** (haemofilus)
- **osteomyelitis** (*Staphylococcus aureus*)
- **cystic fibrosis** (*P. aeruginosa*)
- **burns**
- **IUDs** – can induce infection in pelvis and sepsis
- **artificial breathing** – on surface of tubules – can reach bronchus and lungs
- **intravenous catheter** (coagulasenegative staphylococci)
- **artificial heart valves**
- **joint replacements**
- **contact lenses** – by incorrect handling *Pseudomonas aeruginosa*



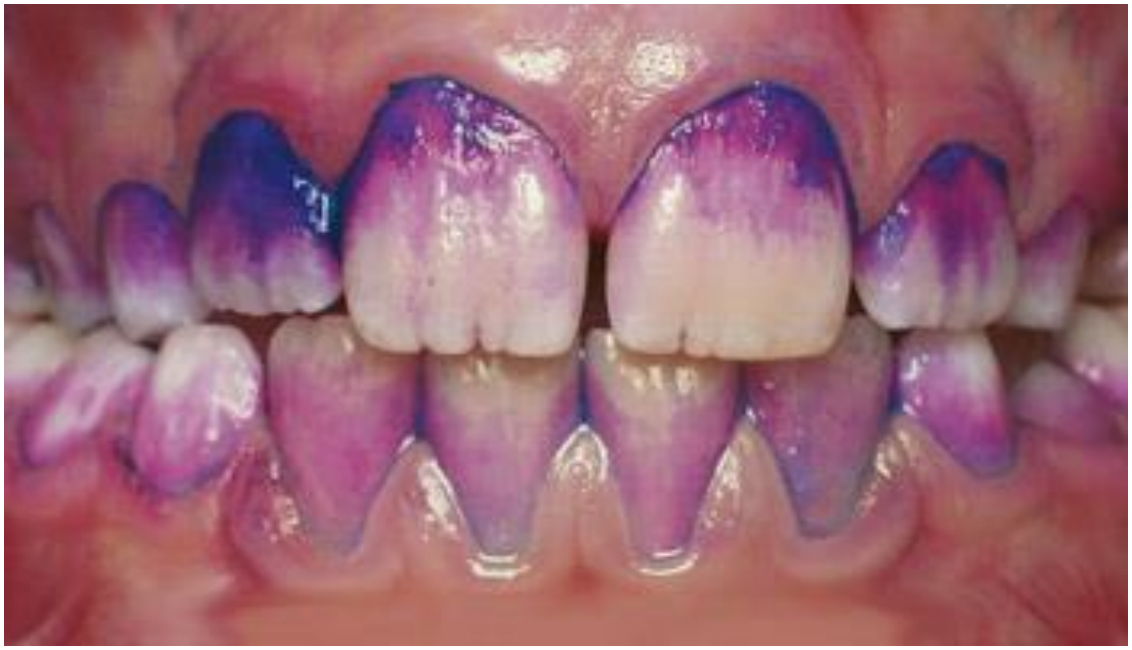
Recommendation

- Determination of MBEC for bacteria forming biofilm is much more suitable than determination of MIC
- In patients irresponsive to therapy is appropriate to determine synergic effect of combination of ATBs
- When microbes are resistant also to combination of ATBs, then only option is to take out the biofilm source (catheters, joint replacements, etc.)
- Prevention: catheters coated with antimicrobial substances e.g. minocyclin and rifampicin, flushing, hygiene.

MBEC= (minimum biofilm eradication concentration) - 4x-1024x higher than MIC

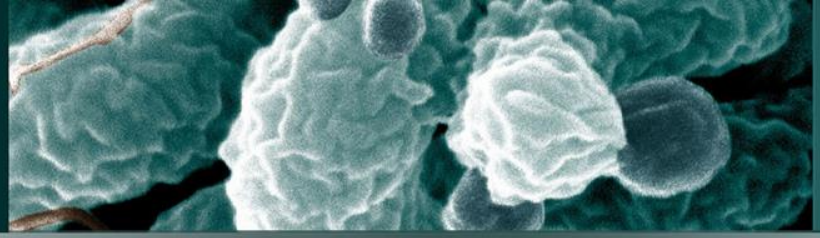


Oral plaque



Old – **BLUE**

Newly formed – **PINK**



Questions for test:

- **What is the difference between primary and secondary resistance**
- **Secondary resistance can develop by two means, name them**
- **Name two microorganisms, in which secondary resistance was developed to an ATB**
- **Name three mechanisms of resistance**
- **Name some of the common mistakes in ATB therapy**
- **Explain what these abbreviations CA-MRSA, HA-MRSA, LA-MRSA stand for**
- **What is synergic effect, name an example**
- **What is biofilm, name an example**
- **What does MBEC mean?**
- **Name some of the places in human body, where biofilm can be formed**