Bacterial cell

Jan Tkadlec

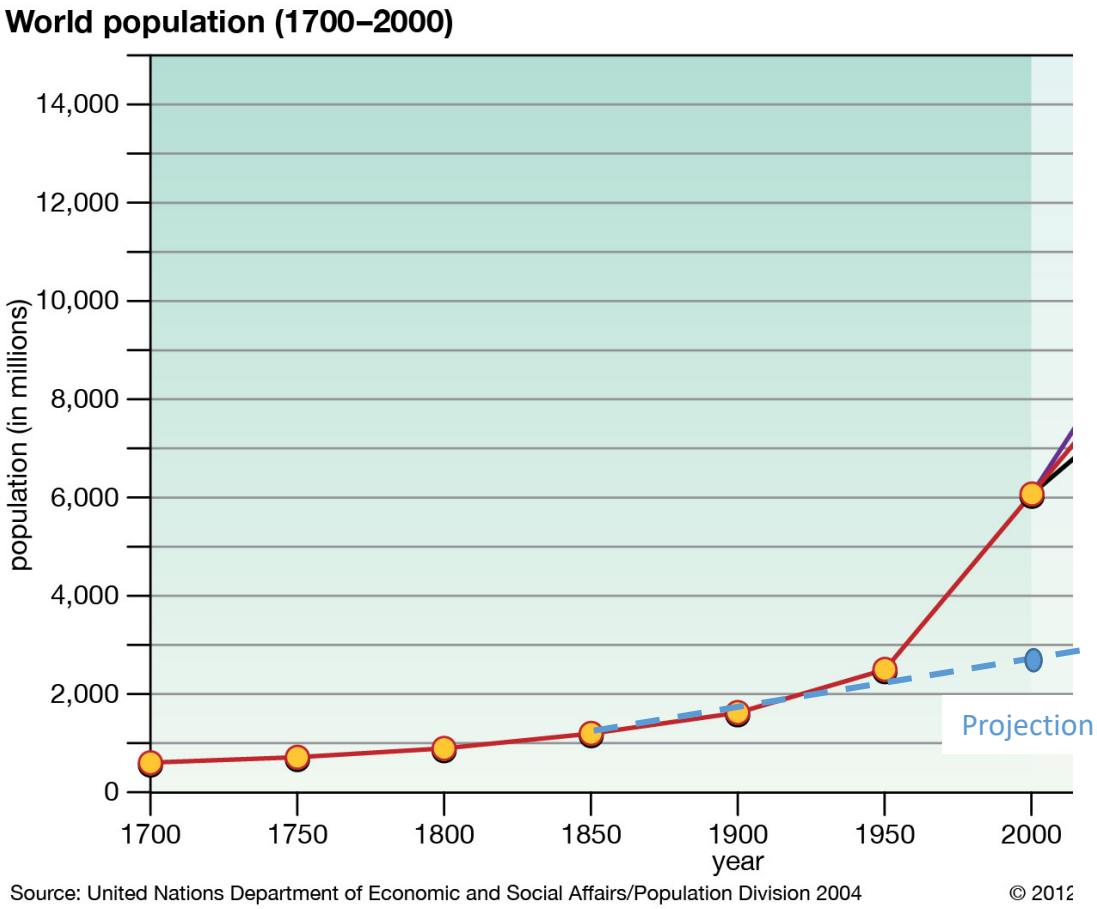
Crucial impact of microbiology on medicine

Surgery almost imposible
Major types of advanced medical procedures are amputation or dissection

Surgery (cardio, neuro, plastic, …)
Transplantation (heart, lungs, …)
Oncologic treatment (chemoterapy, radioterapy…)
Immunosupresion

Modern medicine will be imposible without knowledge of antibiotics, antiseptics, disinfection and microbes as a cause of disease

Understanding microbes: rise of human population



Size of human population: 6 billions in 2000

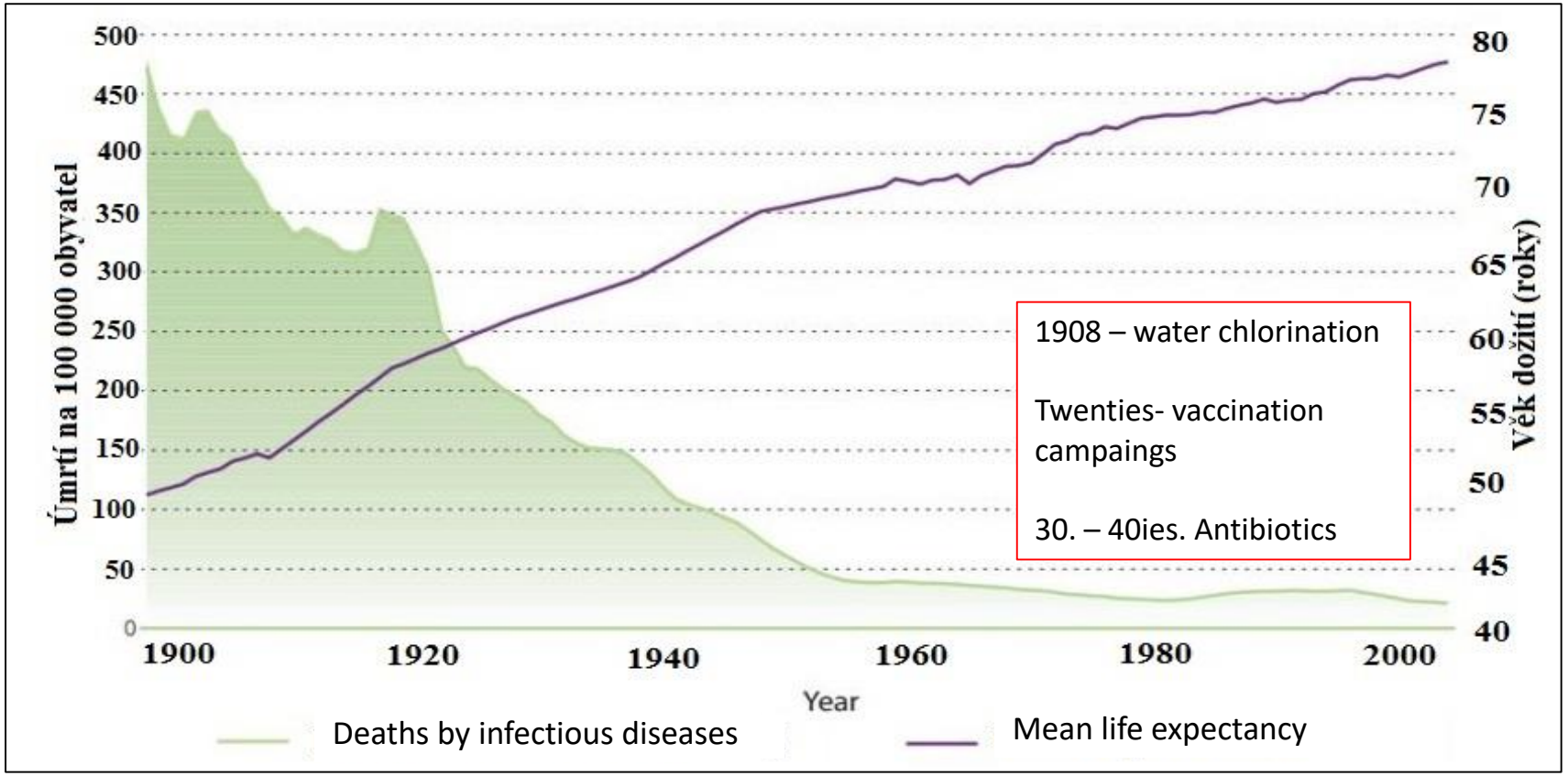
Efect of antibiotics, improved hygiene etc.

Projection based on previous trend: 3 billions

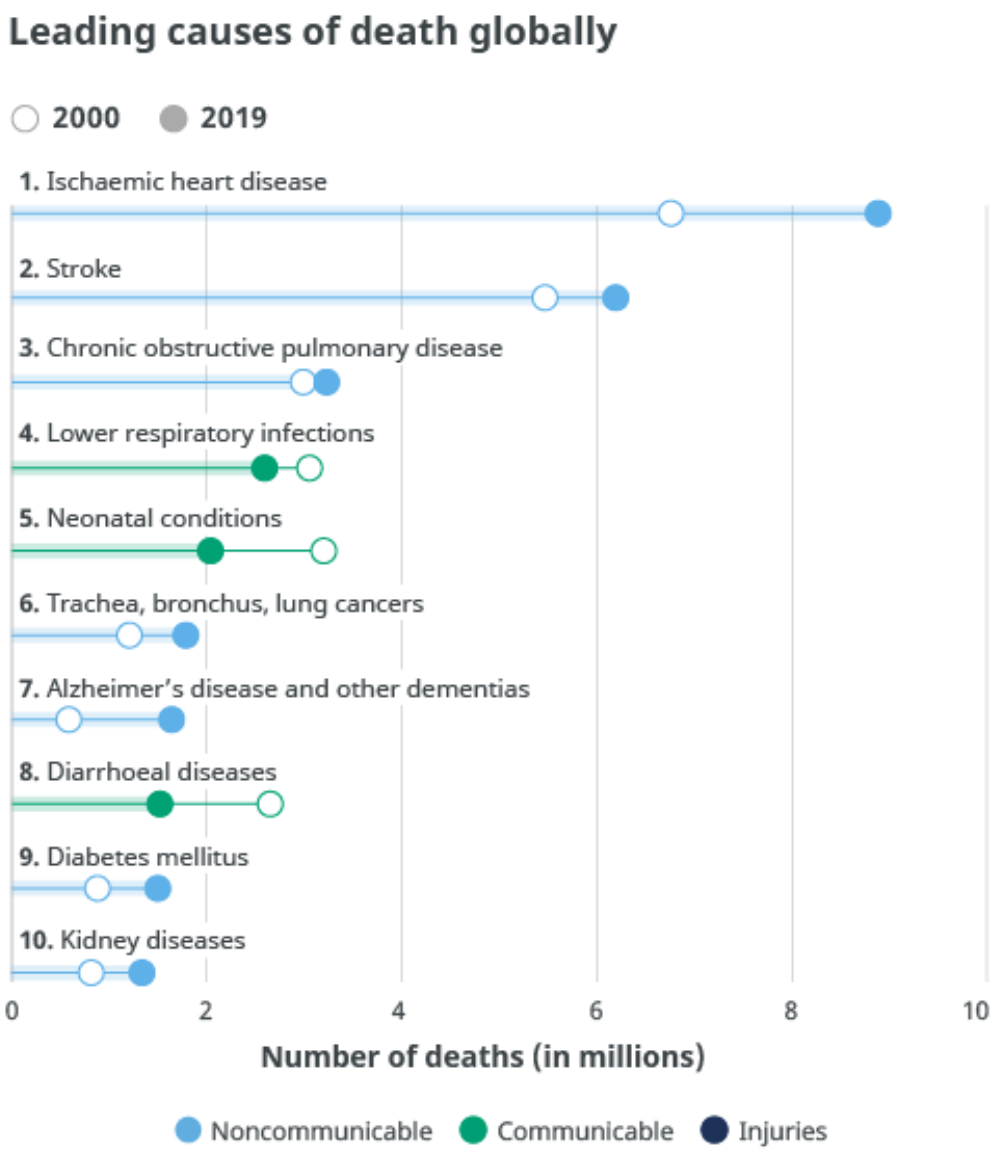
Penicilin itself saved more than 200 millions lives

Is there any other scientific discipline that could claim to save so many lives?

Understanding microbes: increasing life expectancy



Cause of death per year:



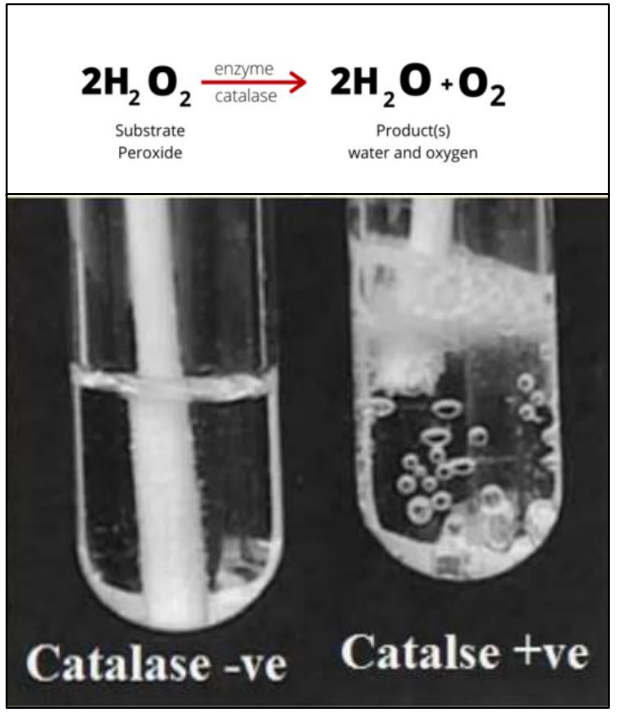
Total 13.7 milions infection related deaths in 2019
Lancet 2022; 400: 2221–48

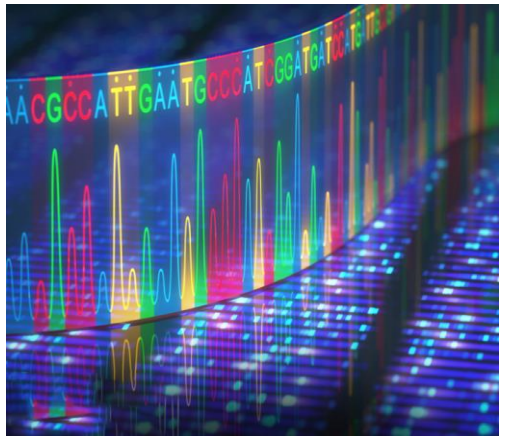
Source:
https://ourworldindata.org/causes-of-death-treemap

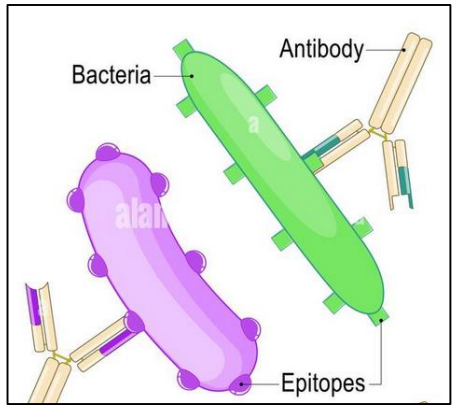
Bacterial cell

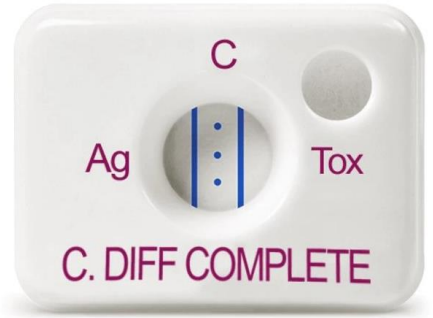
What is interesting on bacterial cell? Medical point of view

1. Features/parts of bacterial cell that could be used for detection or identification of bacterial patogens



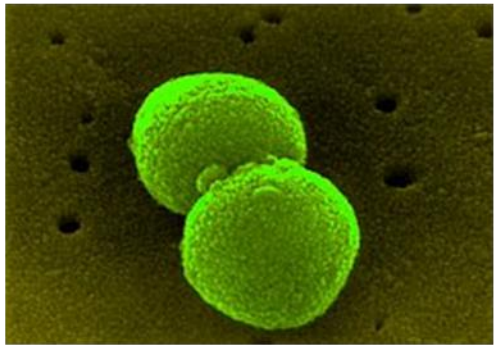




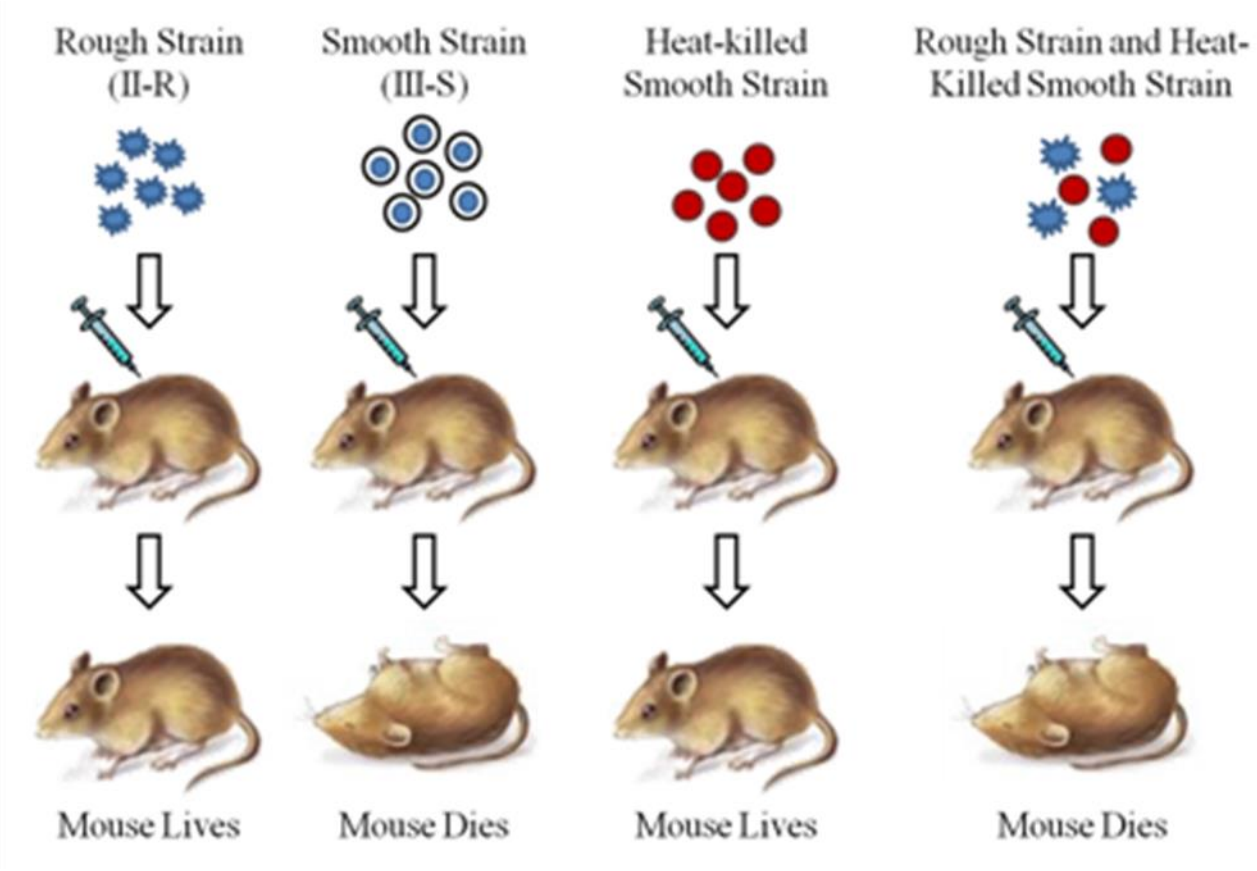


What is interesting on bacterial cell? Medical point of view

2. What gives them ability to cause harm to human body



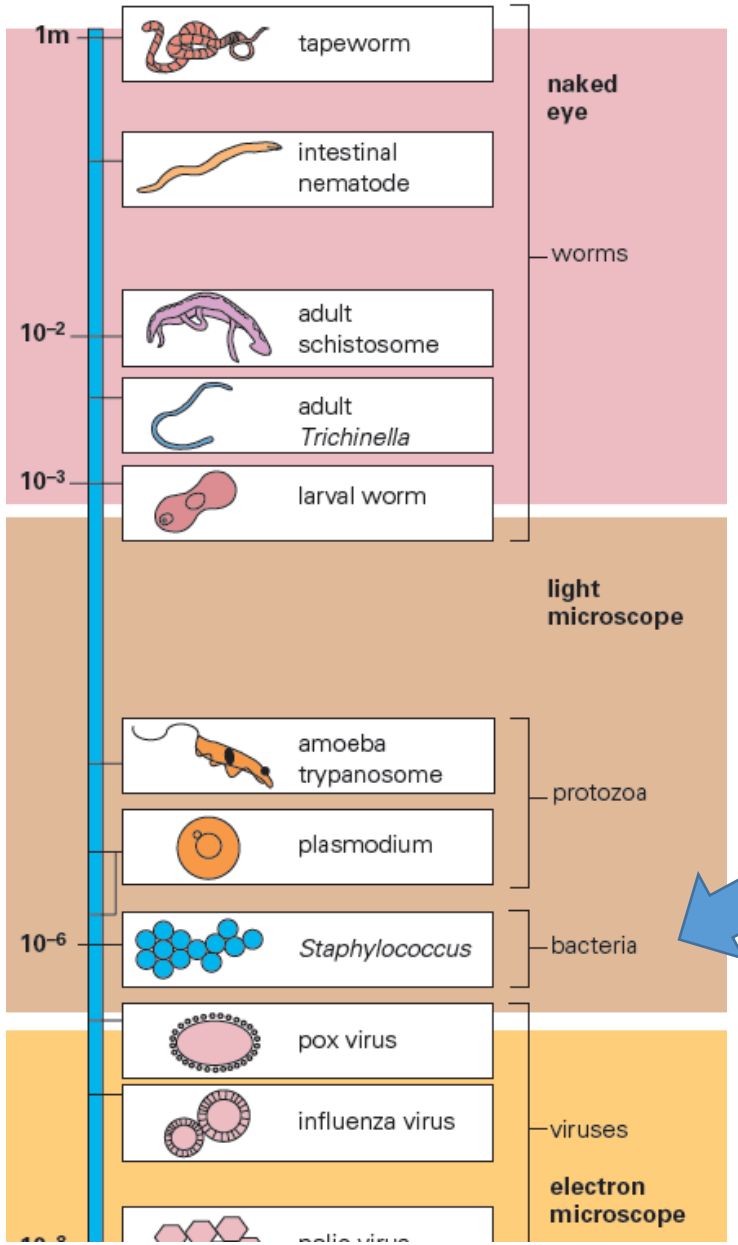
Streptococcus pneumoniae

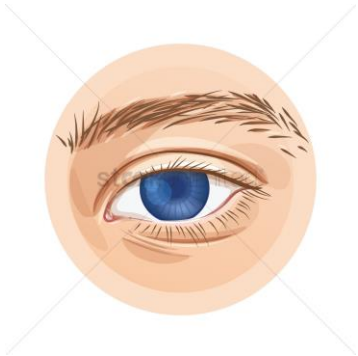


What is interesting on bacterial cell? Medical point of view

3. How to kill bacteria without (to much) harm to the host

Size of human patogens

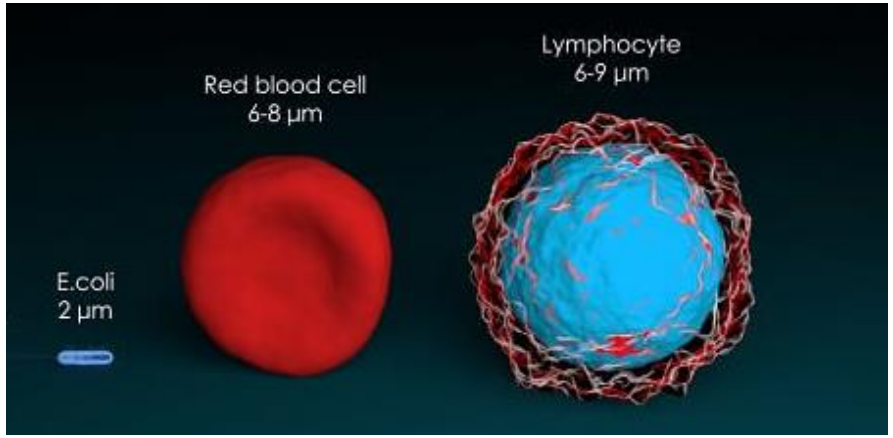


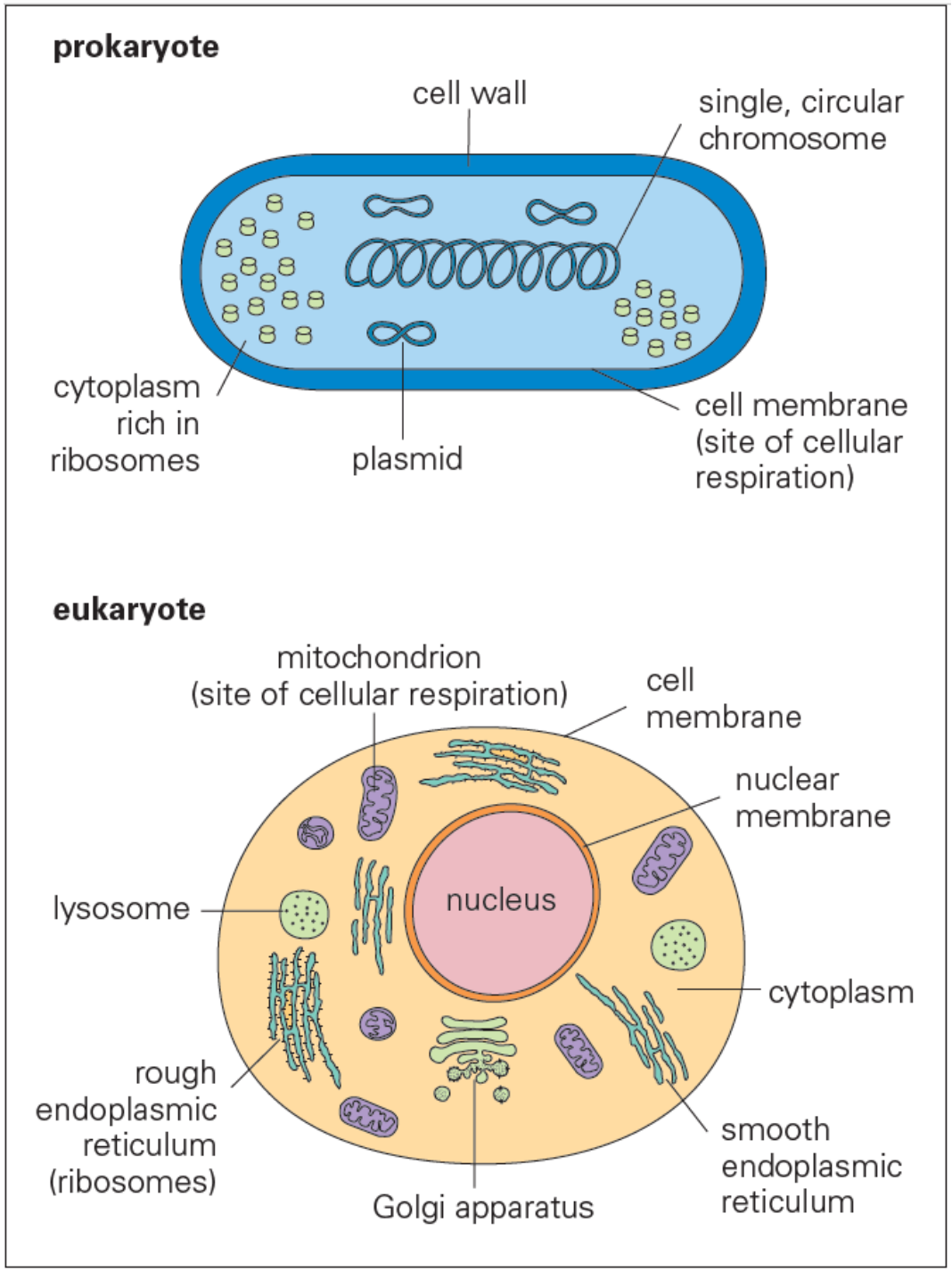


Bacteria are define by their small size! They are microbes.

Size in micrometers (µm)

Light **micro**scope
Direct observation of stained bacteria





Eu vs prokaryotes

Eu

Chromosome separated by membrane

Nucleus

Organelles:

- Mitochondria
- Golgi
- Endoplasm. reticulum
- Lysosome

Eu. flagellum

Eu. ribosome

Pro

Circular chromosome (one copy=haploid)

Plasmids

Cell wall of peptidoglycan

Outer membrane

Bacterial flagellum

Bact. ribosome

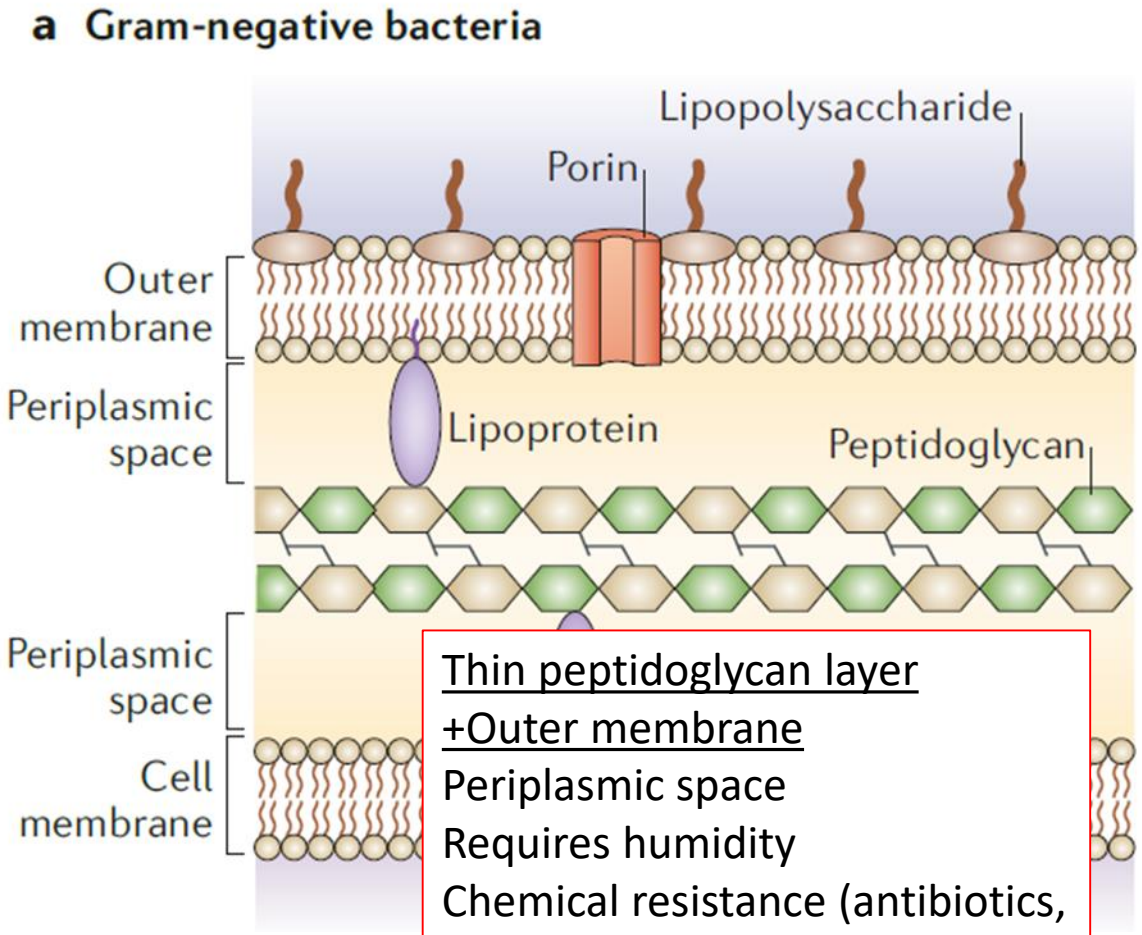
Both

Cytoplasm

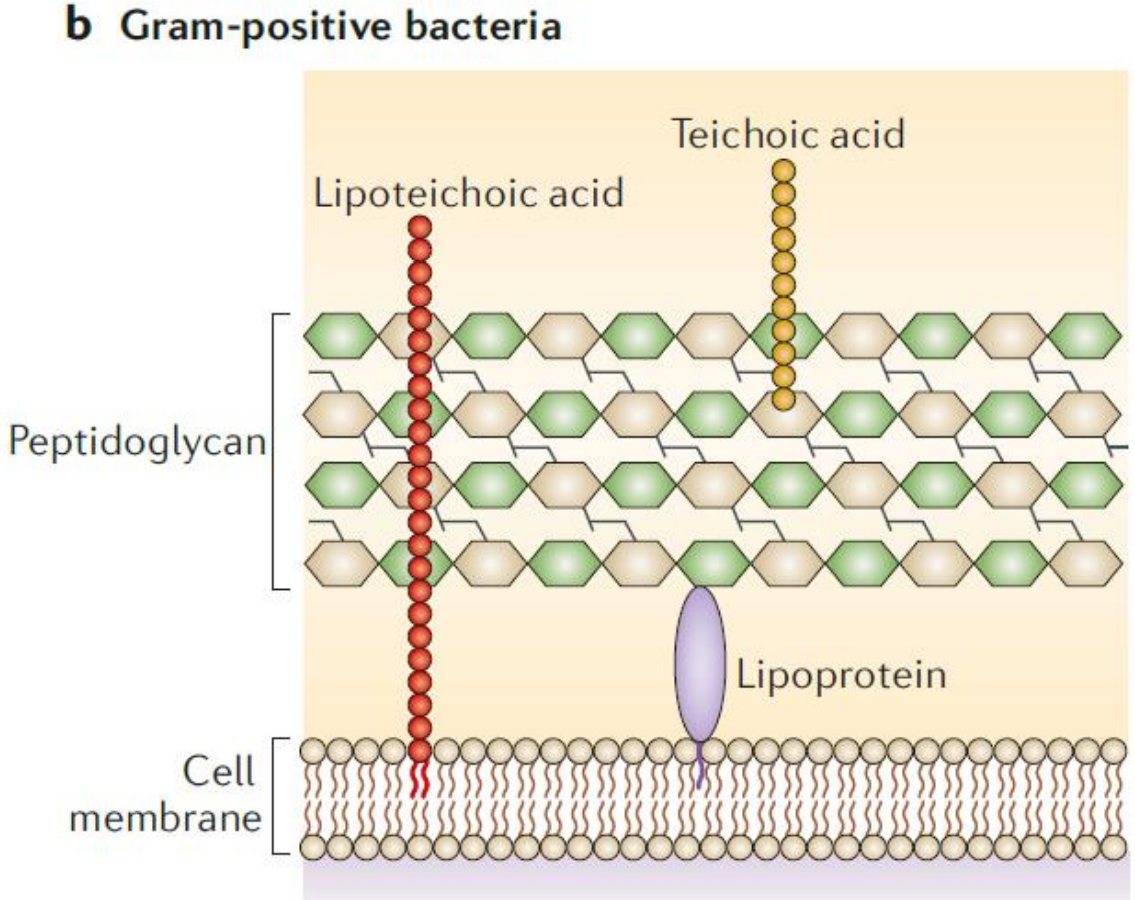
Plasmatic membrane

Structure of cell wall

- Two basic variants



Thin peptidoglycan layer
+Outer membrane
Periplasmic space
Requires humidity
Chemical resistance (antibiotics, enzymes, toxines)



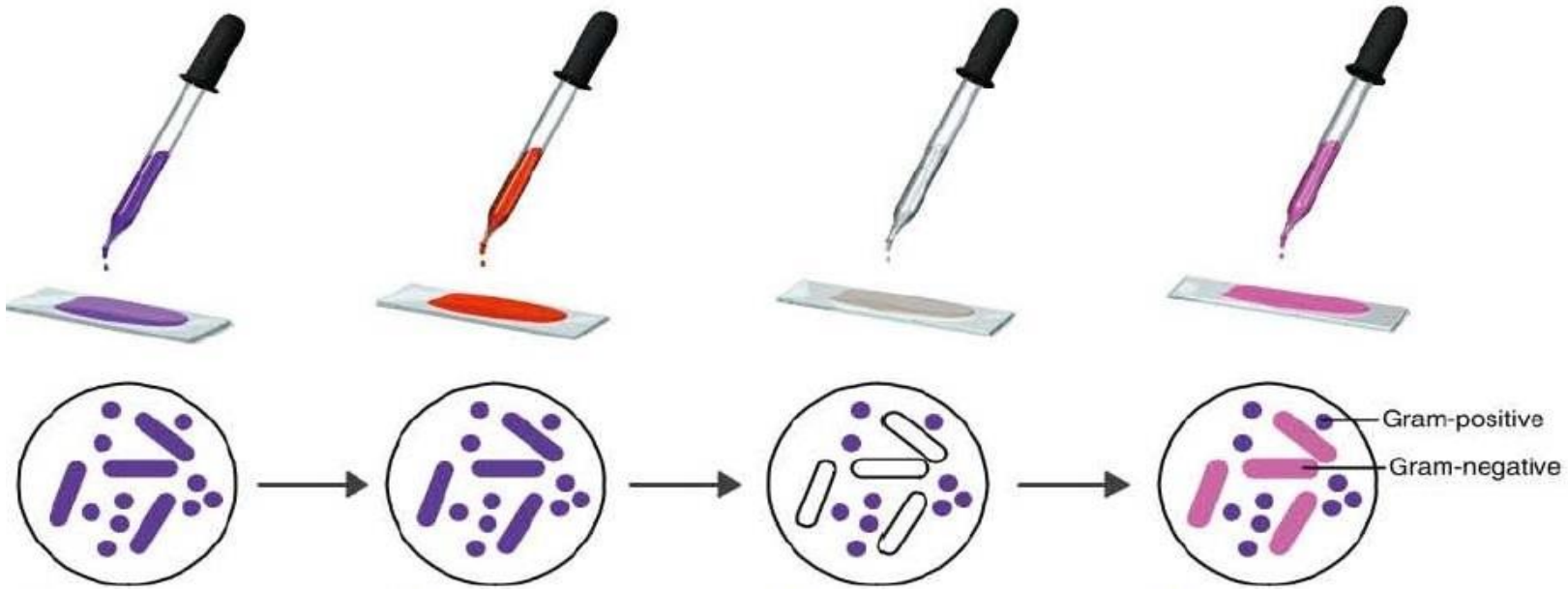
Thick peptidoglycan layer
Terestric bacteria
Physical resistance (desication, irradiation)

Brown et al 2015 Nat Rev Microbiol

Gram staining – most important staining in microbiology

Allow to visualise and distinguish bacteria in clinical sample

Procedure:

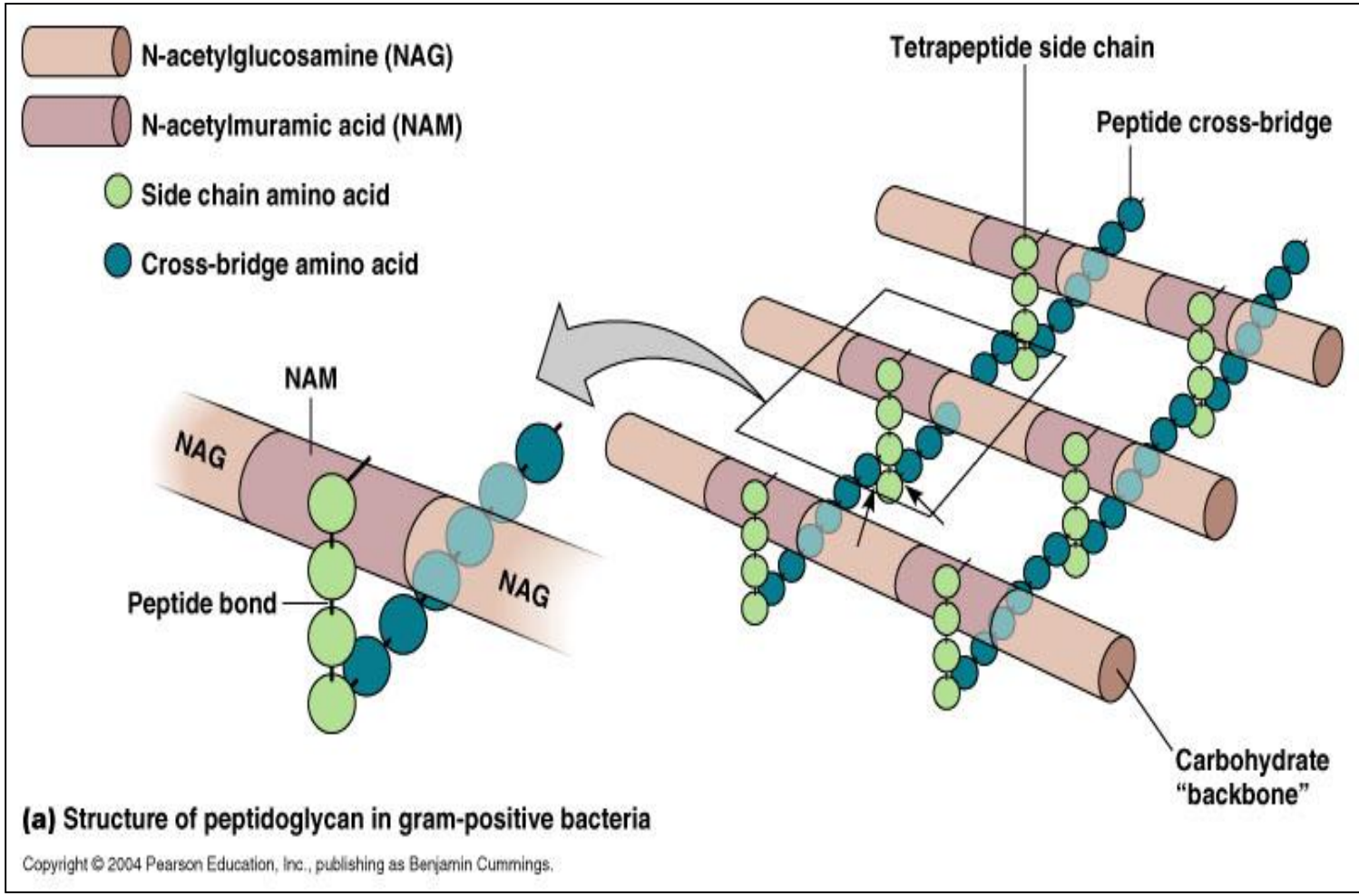


Violet **L**ugol **A**lcohol **K**arbolfuchsin

Vlak = train

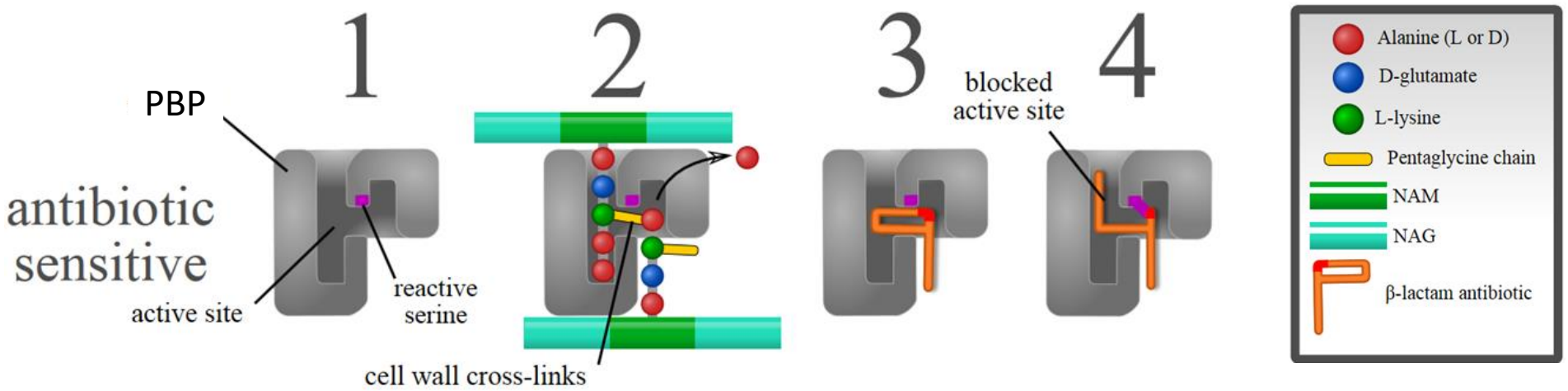
Peptidoglycan

- Only in bacteria
- Rigid but permeable
- Integrity and shape of bacterial cell
- Protects from
  - irradiation, desiccation, mechanical damage, inner pressure
- Polysacharide:
  - N - acetylglucosamine
  - N - acetylmuramic acid
- Polymer fibers croslinked via peptide side chain into net like structure



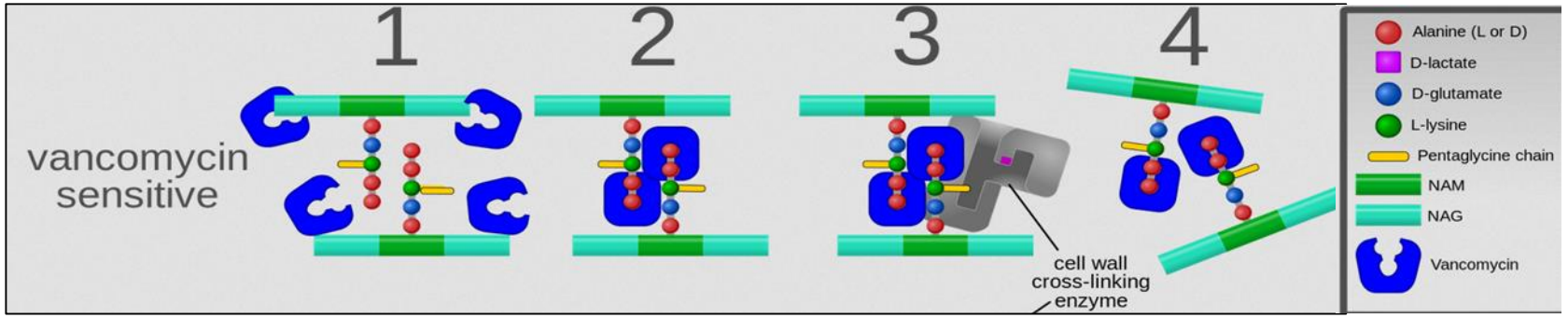
(a) Structure of peptidoglycan in gram-positive bacteria

Peptidoglycan synthesis – target of antibiotics



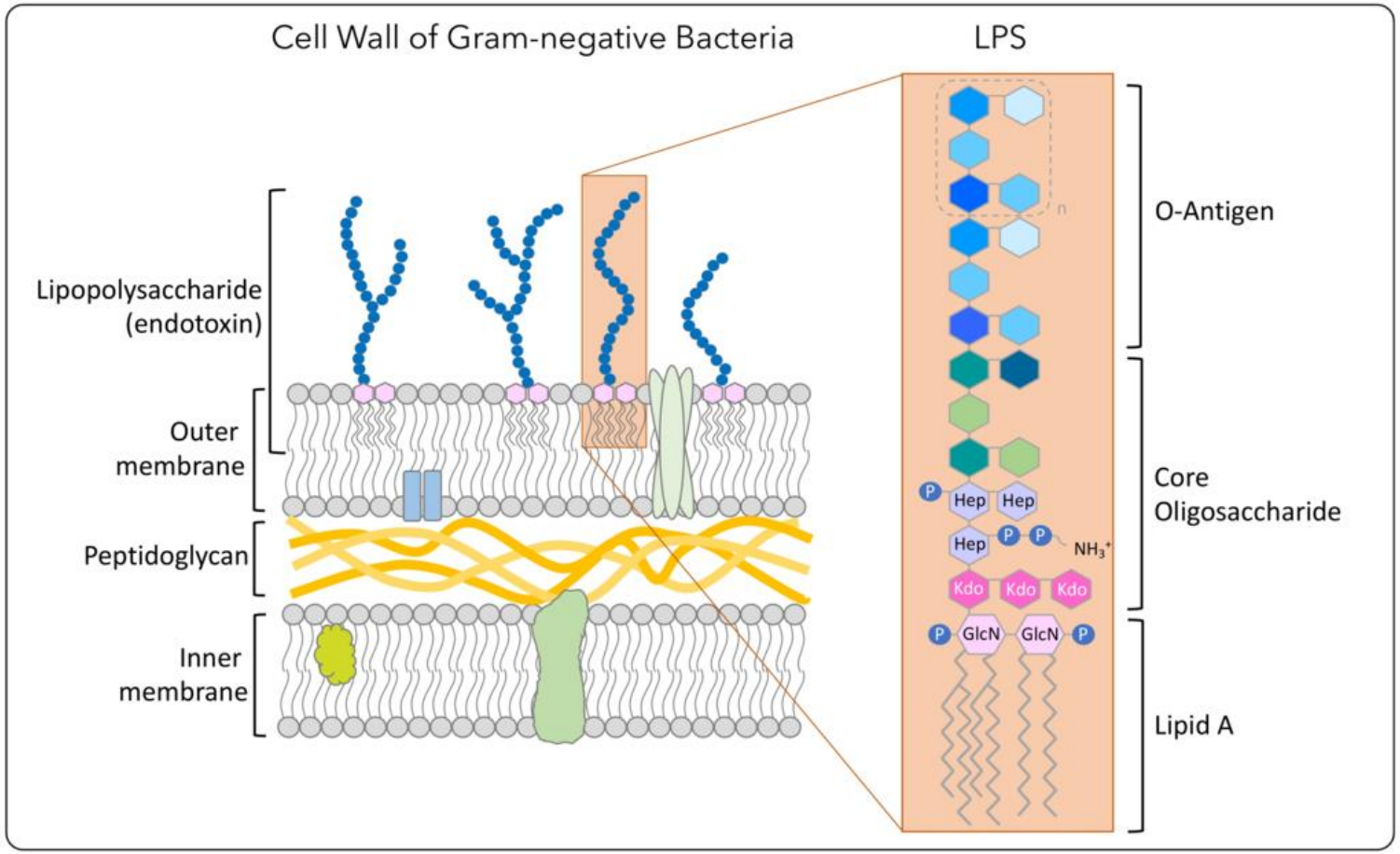
PBP – penicilin binding proteins - transpeptidases cross-link of peptidoglycan fibers – main target of **antibiotics**

- **Beta-lactams** (penicilins, carbapenems) and **cefalosporins** binds and inhibits PBP
- **Glycopeptides** (vancomycin, teicoplanin) binds aminoacid side chains and prevents PBP binding



Inhibited action of PBP cause lysis of the cell when growing.

Outer membrane



<u>Only Gram negatives</u>

Lipopolysacharide (LPS)

<u>Endo</u>toxin = part of the cell

- O-antigen
  - Highly variable – e.g. serotyping *E. coli* **<u>O157</u>**:H7
- Core polysaccharide
- Lipid A – toxic part of LPS

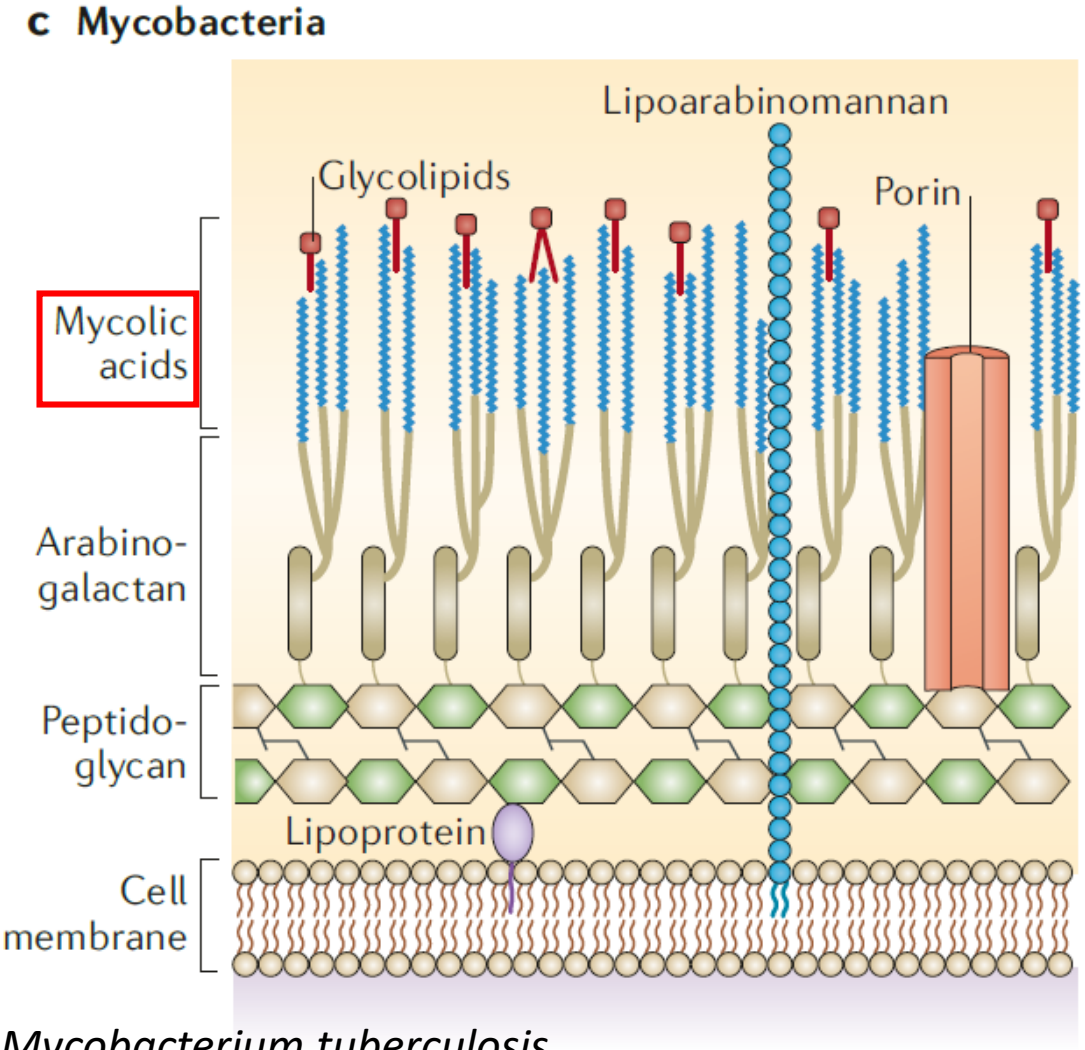
LPS is PAMP (**P**athogen-**A**ssociated **M**olecular **P**attern)

Molecule specific for bacteria recogised by innate immunity (complement, macrophages)

Septic shock

Outer membrane is target of antibiotics: polymyxins (colistin)

Mycobacteria – Gram resistant bacteria

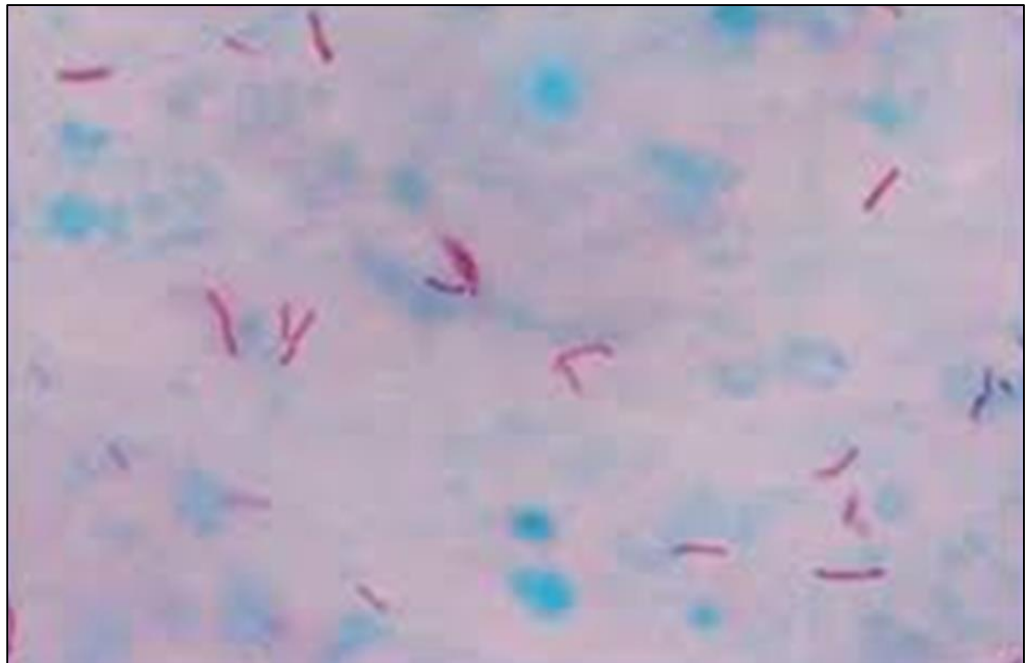


Mycobacterium tuberculosis
M. leprae

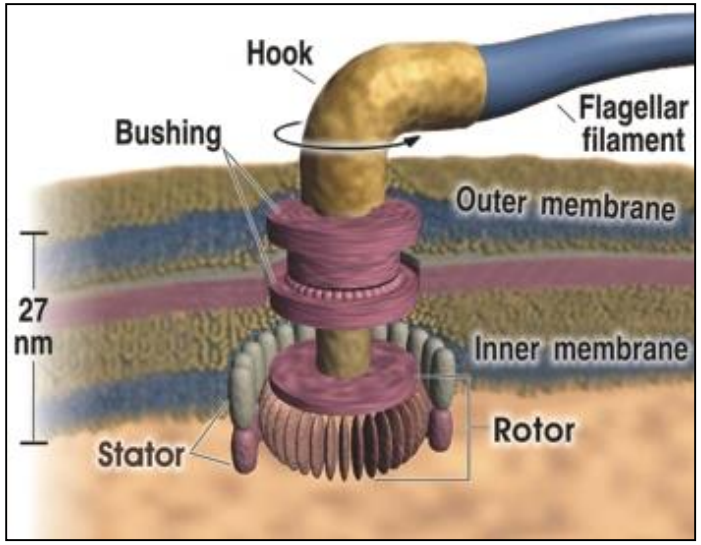
High content of wax-likes compounds - mycolic acids
Repulse stain and alcohol
Cause of high physical and chemical resistance of mycobacteria

- Antibiotic resistance

Fast acid or **Ziehl-Nielsen staining**

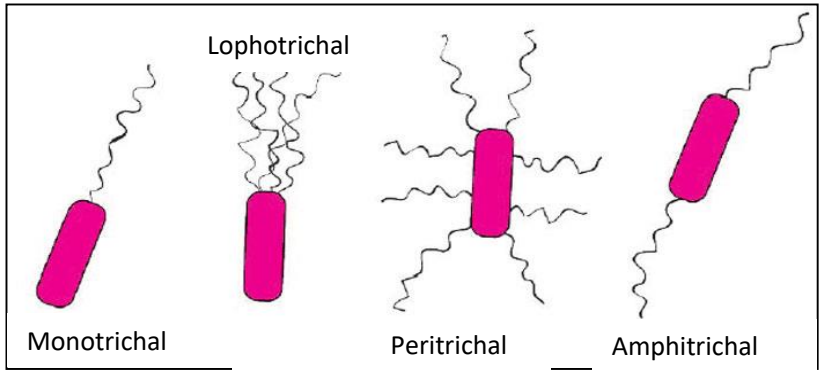


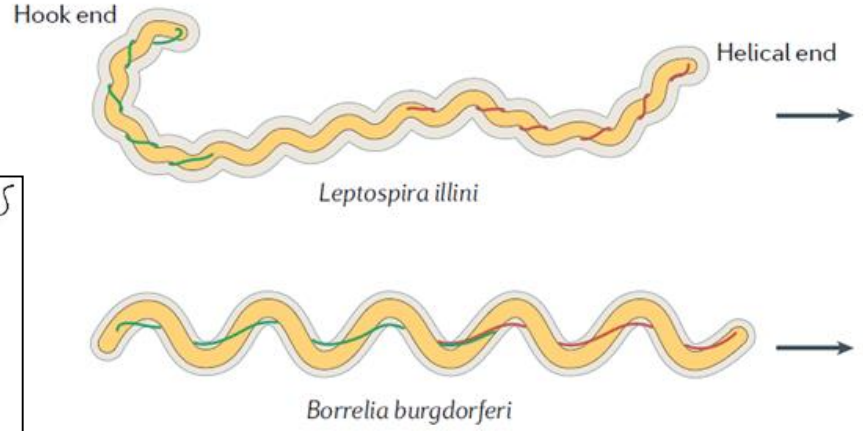
Bacterial flagellum



- Anchored in the membrane
- screw-propeller like mechanism
- Energy from proton gradient
- Some bacteria lacks flagella (streptococci, staphylococci)
- Spirochaetes flagellum (=axial filament) corkscrew like motion
- **Highly antigenic**
  - Serotyping of enterobacteria: **H antigen**

Different arrangement

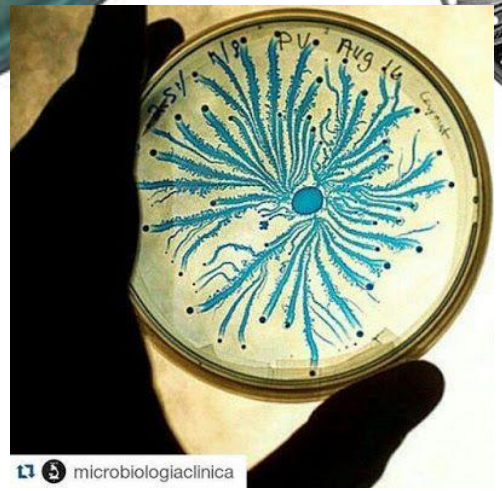


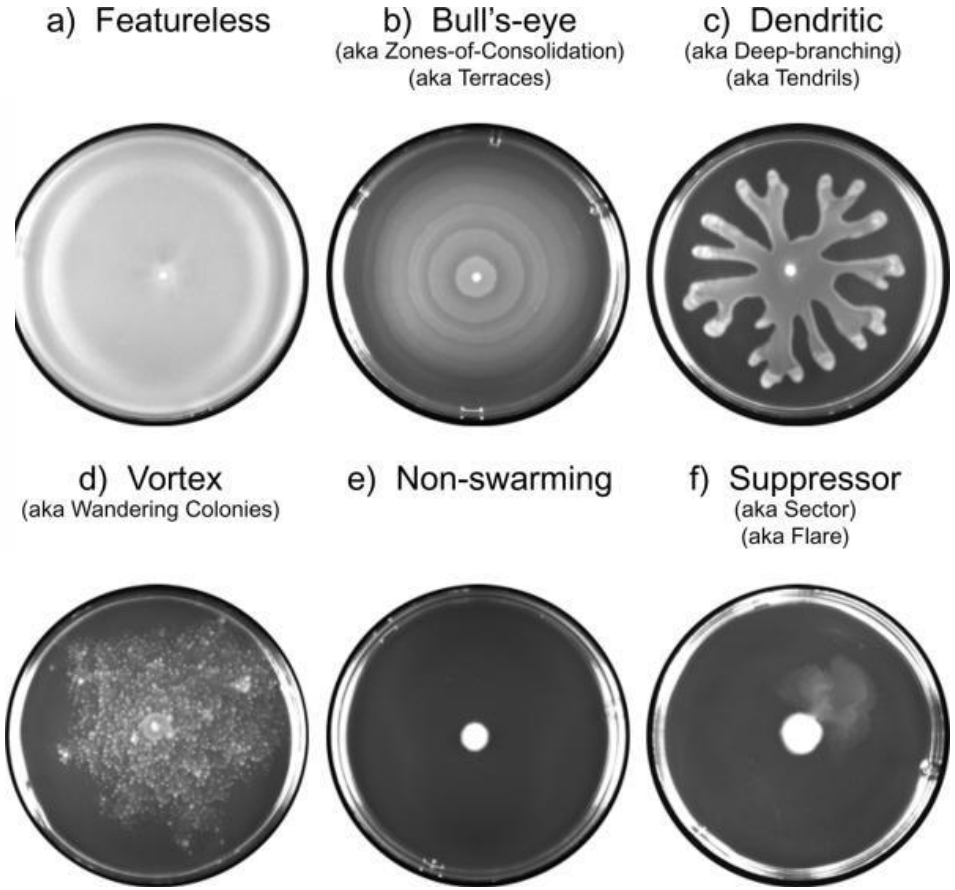


Movement on the solid surface

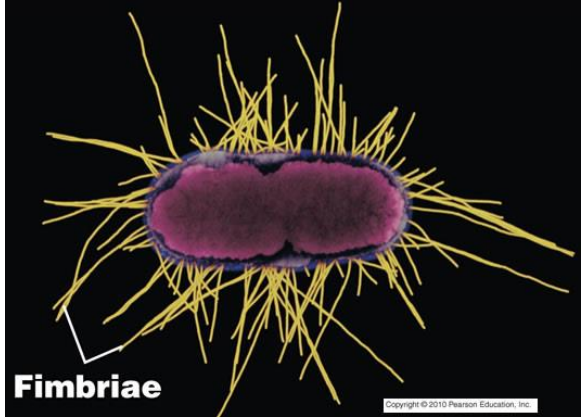
Swarming e.g. *Proteus*

- CLED agar – inhibition of swarming
  - Otherwise *Proteus* will overgrow other bacteria





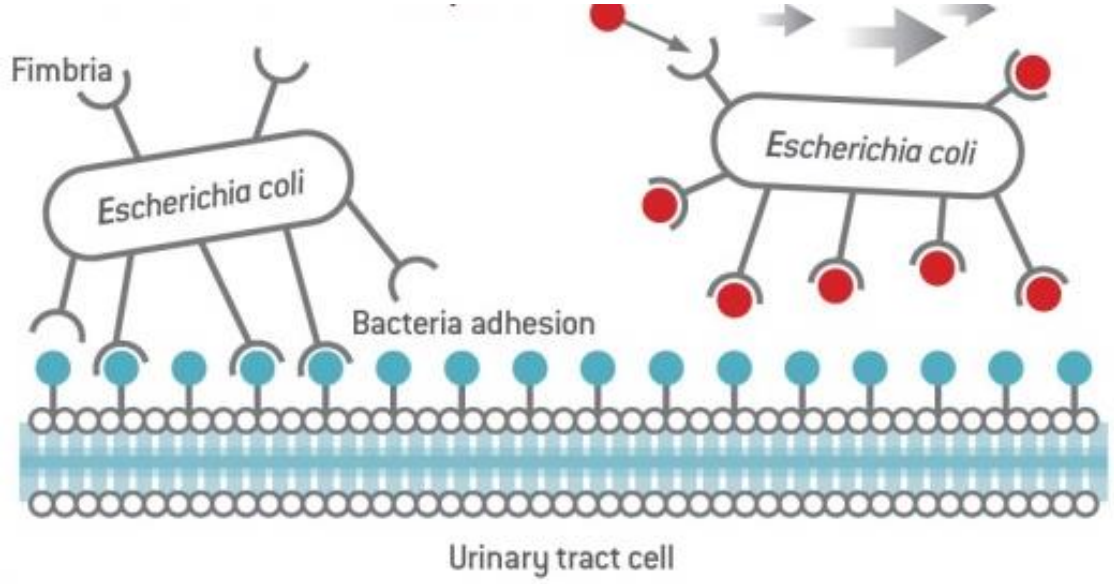
Fimbria

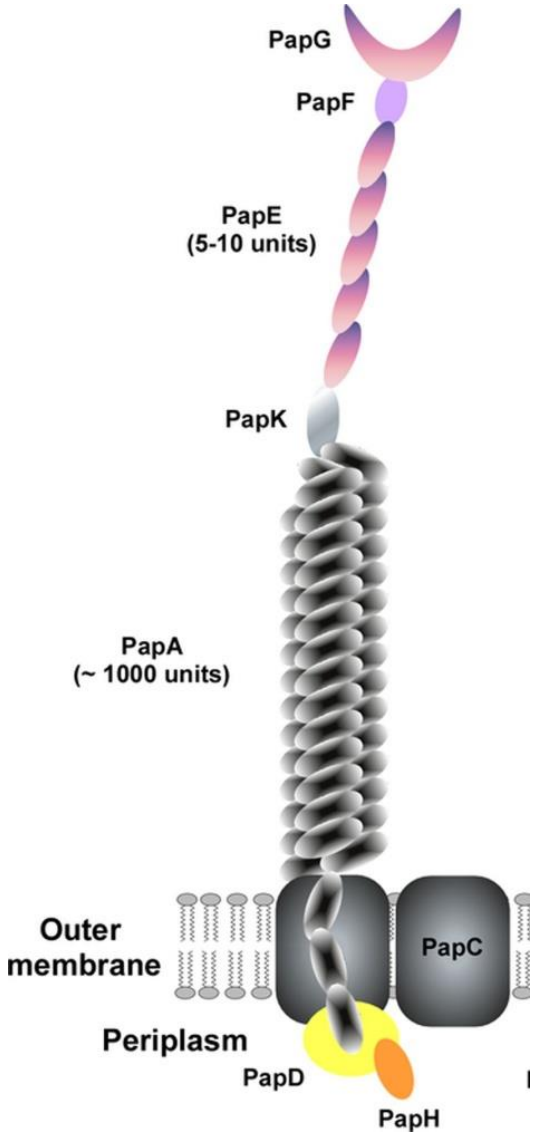


- synonym pili (pilus)
- Shorter than flagella, around the surface of the cell
- Made of proteins (like flagella)
- Sex pili – conjugation (plasmid transfer)
- **Main function is adhesion**
  - **adhesins** on the top of the pili
  - adhesin bind specific sacharide on the surface of human cells
  - **Tissue specific**

Fimbria

- *Escherichia coli* urinary tract infection
  - PAP fimbria (**P**yelonephritis-**A**ssociated **P**ili)
  - Natural remedies: cranberries
  - Saccharides from cranberries goes into urine and blocks adhesins on fimbria

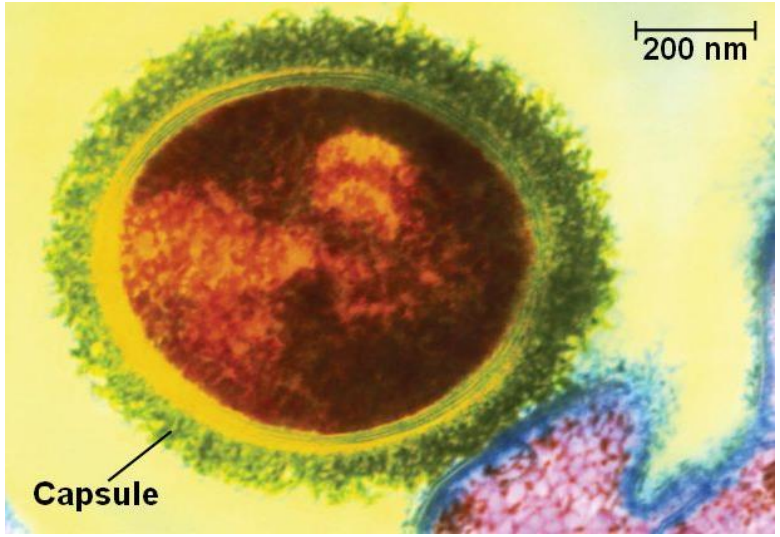




Structure of pili

Capsule

- Additional protective layer – polysacharide
- Important for adhesion
- Protection from desication and **<u>immune system</u>**
  - Hides surface antigens (PAMPs) from phagocytes, complement and antibodies
  - Antigenic variation
- Frequently found in serious patogens
  - *Neisseria meningitidis, Haemophilus influenzae*, *Streptococcus pneumoniae, Bacillus anthracis* etc.
- Essential for full virulence (pneumococci)
- Vaccination

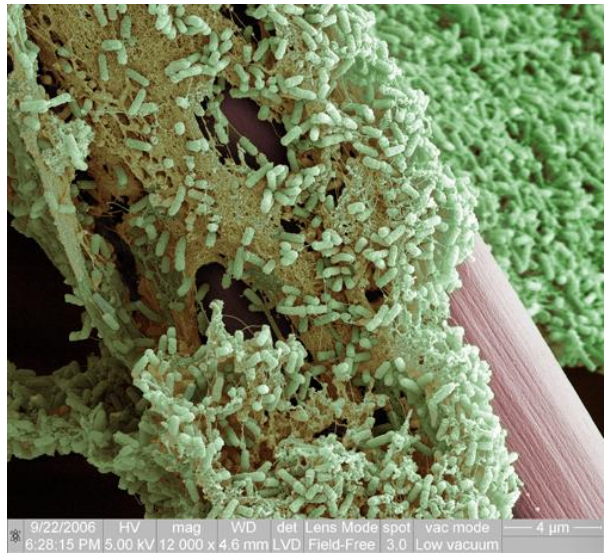


Electron microscopy

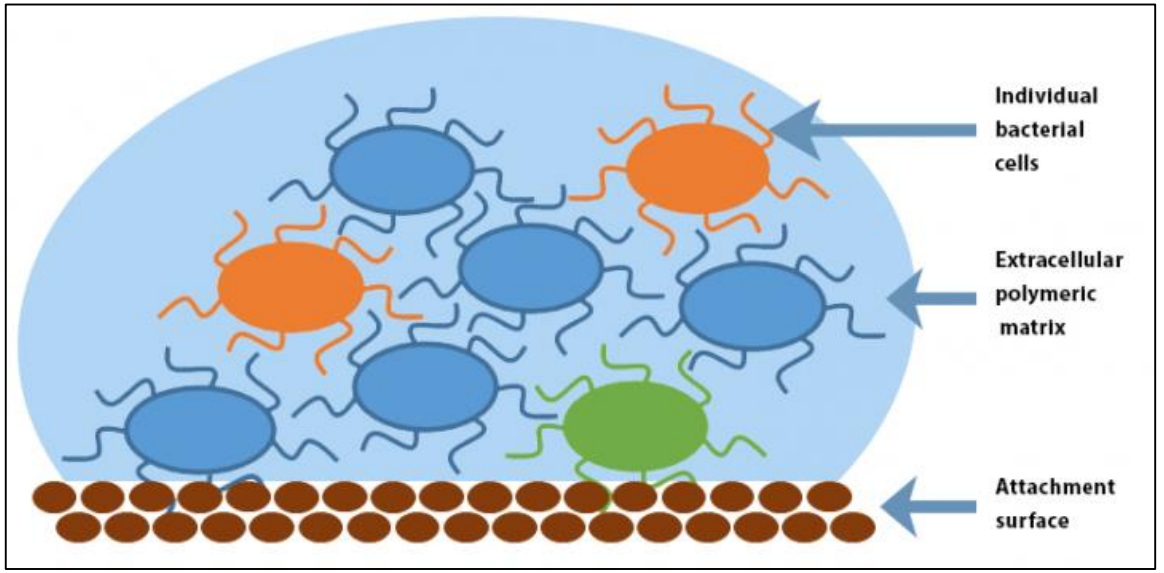
Halo around bacterial cells when stained by bury stain

Biofilm

- Bacteria dont form a tissue
- But they could form a biofilm!!!
- Biofilm is **attached** structured consorcium of bacteria enveloped by **extracellular matrix** from polysacharides, proteins and extracellular DNA secreted by these bacteria

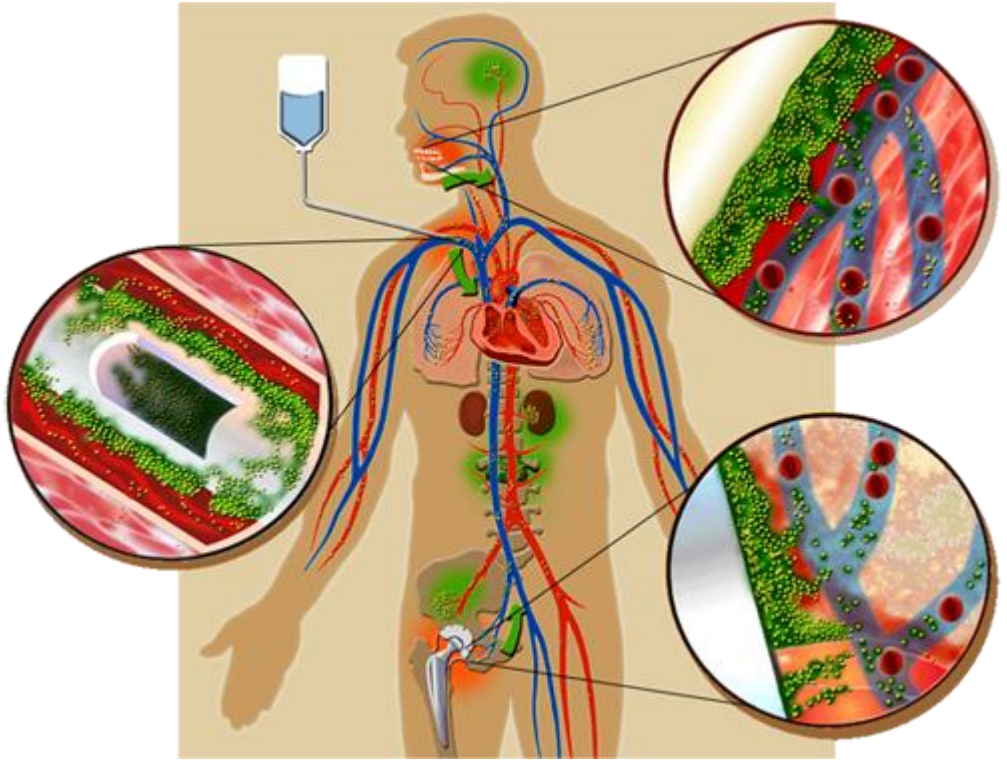


- Base of the biofilm is protected from
  - antibodies
  - complement
  - phagocytes
  - antibiotics
- Regeneration from the base



Biofilm – Clinical impact

- Majority of pathogenic bacteria forms biofilm
- Protects from antibiotics and immune system
- Infection of foreign bodies:
  - **implants**
    - prostetics
    - dental implants
  - **catheters**
    - vascular
    - urinary
  - Dental plaque
- Biofilm is impossible to eradicate



Bacterial ribosom

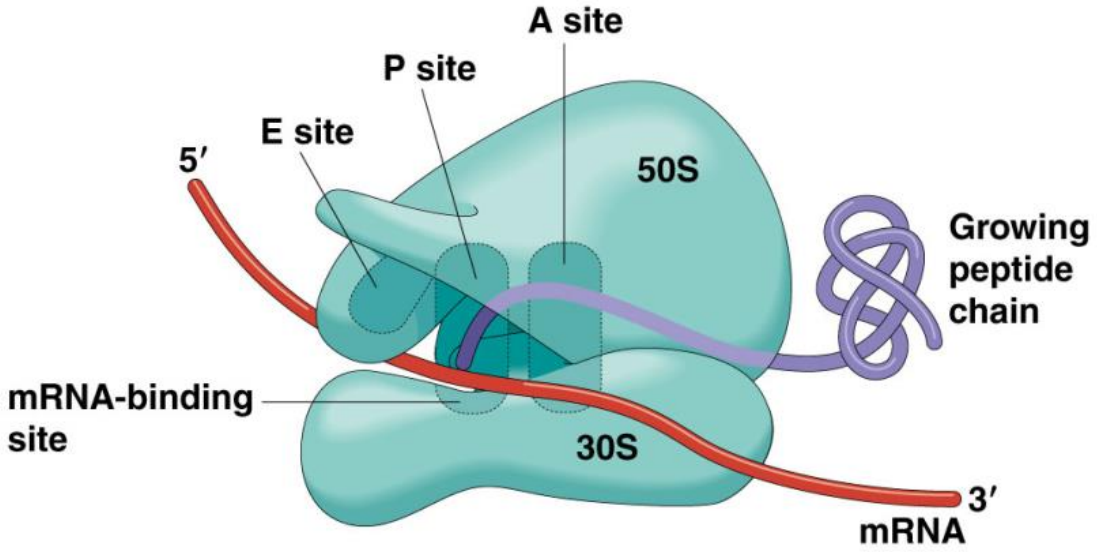
Function – translation= protein syntesis

Size - 70S (S =Svedberg unit of sedimentation)

vs. Eukaryotic 80S

- large (50S) and small (30S) subunit

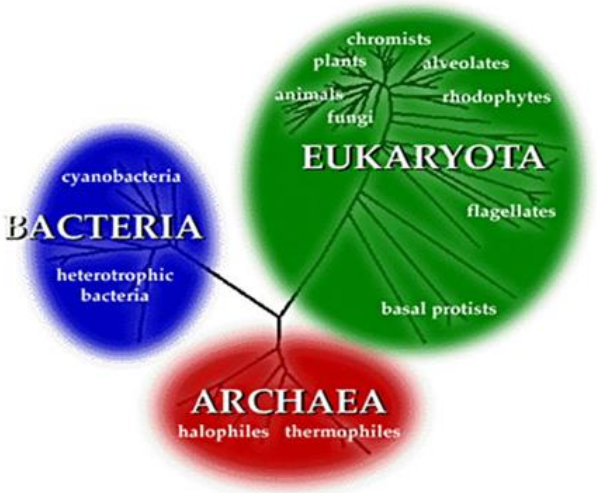
vs eukaryotic 60S a 40S



16S rRNA (18S Eu) – part of small subunit

- In all living organisms
- Sequence – taxonomy, diagnostics

CARL WOESE (1990)

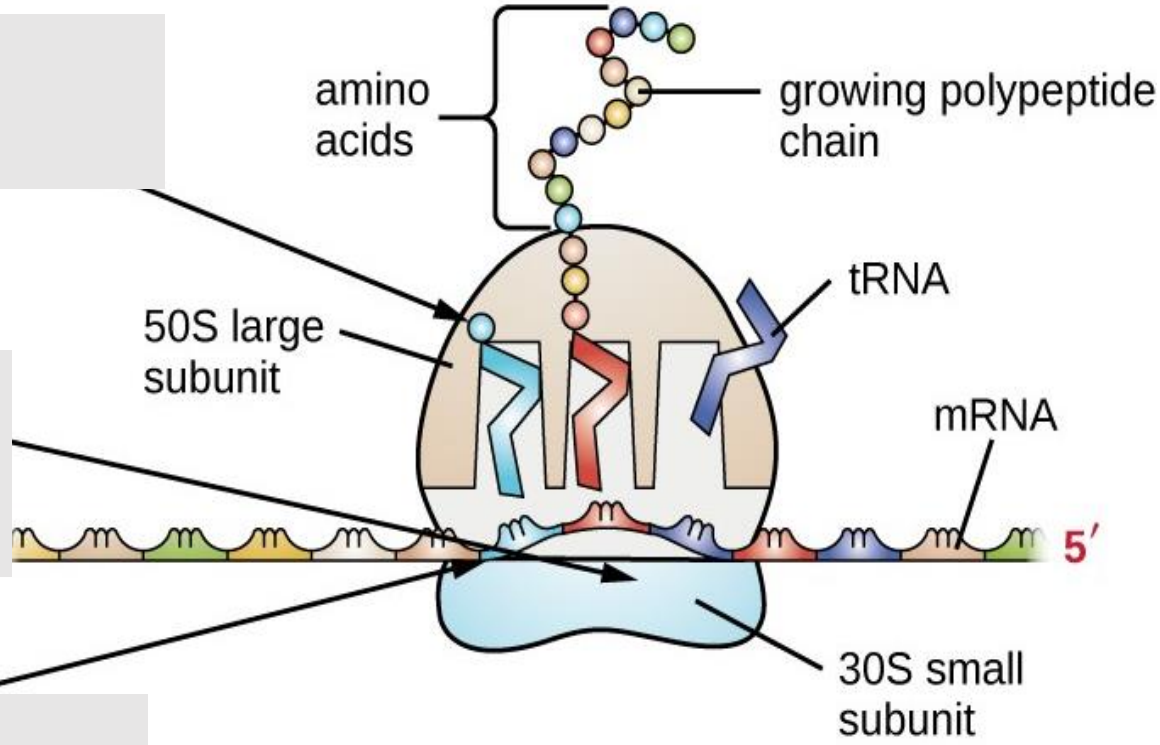


Antibiotics targeting ribosom

Macrolides and linkosamides
- Large subunit
- Block the protein syntesis

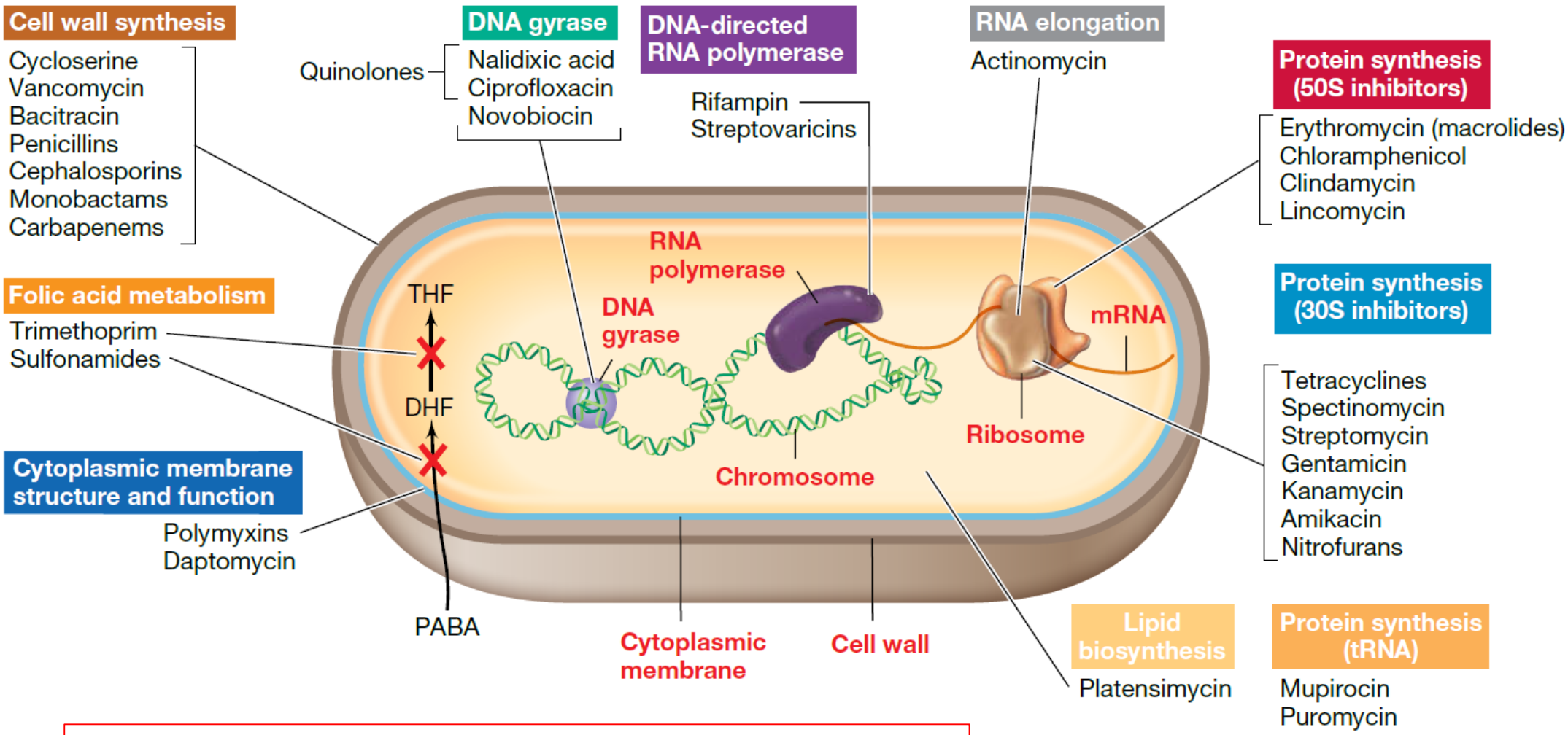
Aminoglycosides
- Small subunit
- accumulation of mistranslated peptides - toxic

Tetracyclines
- Small subunit
- Prevents tRNA recognition



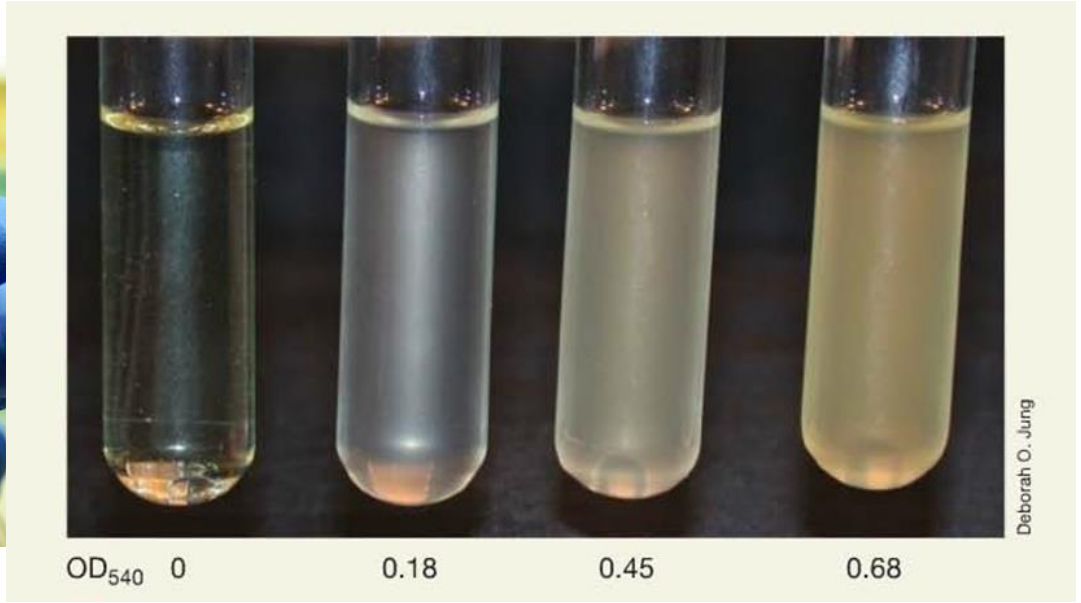
Mostly bacteriostatic effect
Great when toxin production is targett of the treatment

Targets of antibiotics in bacterial cell



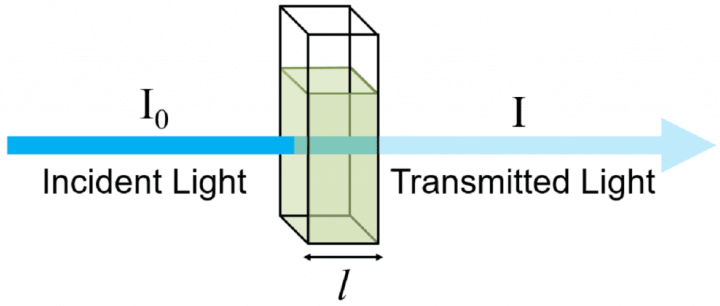
Mostly processes associated with cell growth
Antibiotics targett growing bacteria, not dormant like spores!

Growth of bacteria



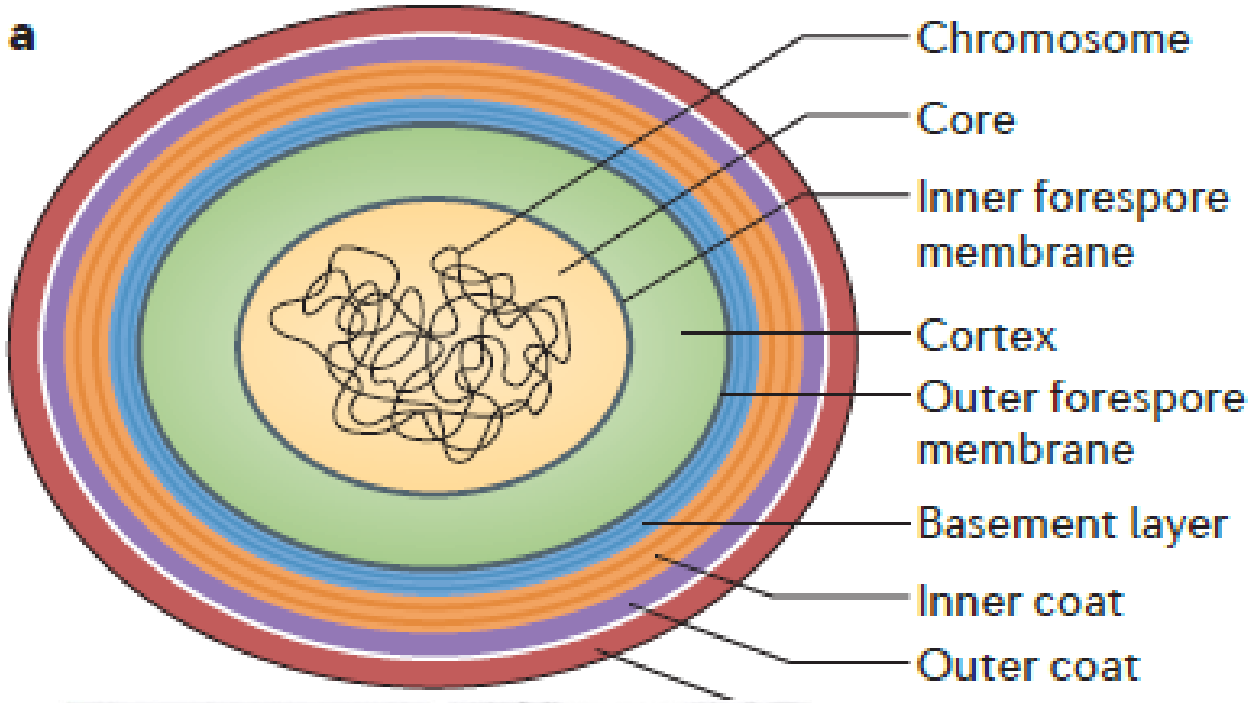
Lambert-Beer law
Turbidity corelates with amount of bacteria

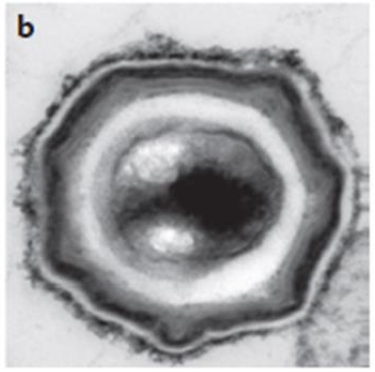
Light source



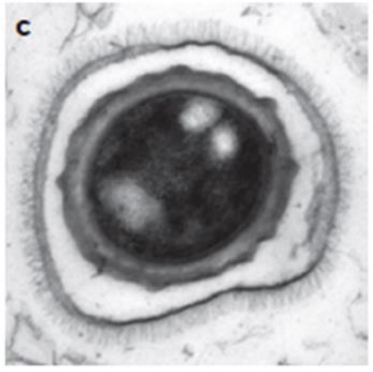
McFarland	0.5	1	2	3	4
Bakterií/ml (x 10^8)	1.5	3.0	6.0	9.0	12.0
OD_{600}	0.08-0.1	0.257	0.451	0.582	0.669

Spora





Bacillus subtilis



Bacillus anthracis

Durable, non-metabolising and dormant form

Concentrated cytoplasm

Multiple protective layers

High chemical (alcohol) and physical resistance (boiling, UV irradiation)

endospores

Highly toxigenic bacteria

- *Clostridium tetanii*
- *Clostridium botulinum*
- *Bacillus anthracis*

McKenney et al, Nature Reviews Microbiology 2013

Bacteria and the oxygen

a) Aerobic (e.g. *Mycobacterium*)

- Requires oxygen
- respiratory metabolism
- Oxygen is final electron acceptor

b) Anaerobic (e.g. *Clostridium*)

- Hates the oxygen (fermentation), oxygen makes them sick

c) facultative anaerobic (most of the bacteria)

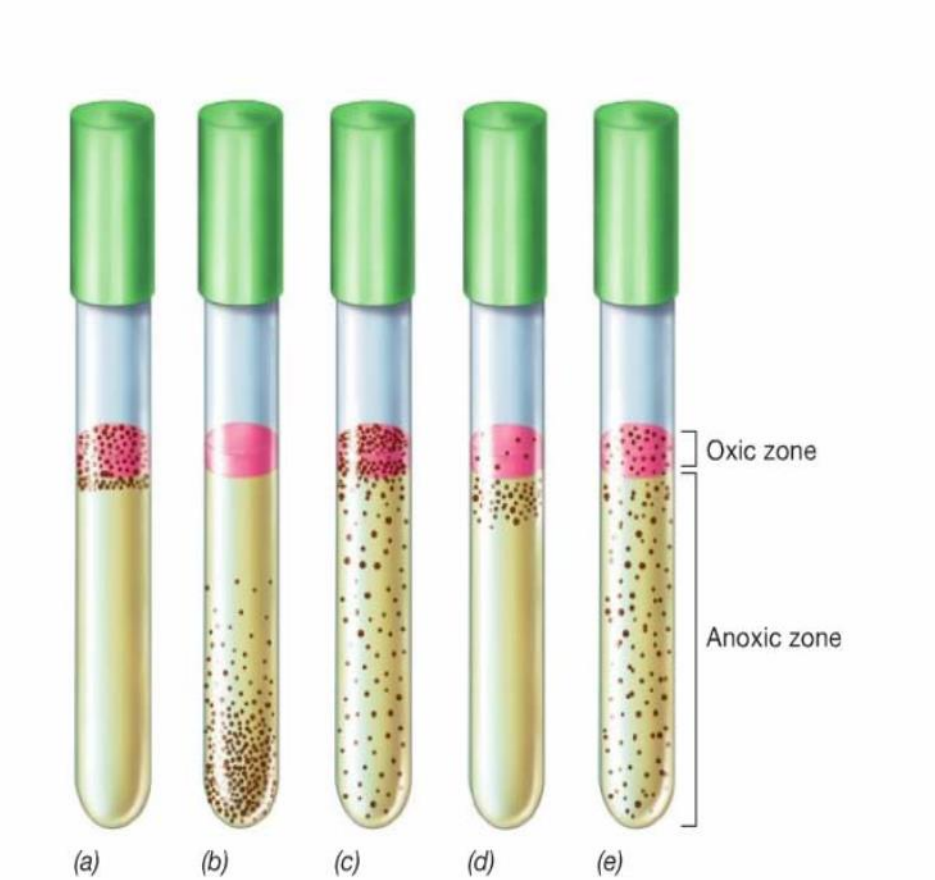
- Prefer oxygen (respiration), but are OK without it (fermentation)

d) Microearophilic (e.g. *Campylobacter*)

- Requires oxygen bur not too much (cca 2 %)

e) Aerotolerant - (e.g.. Streptococcus)

- Dont care about oxygen (fermentation)



Pathogenic bacteria

Pathogenic bacteria

- **Pathogen** - biological factor (microorganism) able to cause disease
- **Pathogenicity** = capacity to cause disease
    - qualitative: pathogenic vs non-pathogenic
    - Feature of the species
- **Virulence** = quantitative measure of patogenity
    - Feature of a bacterial strain
  - Letal dose LD_{50} – death of 50 % of tested subjects in 24 hours from exposition – measured as amount of inoculated bacteria
  - Infective dose ID_{50} – dose capable to cause disease in 50% of tested subjects
  - Depends on the bacterial strain and host

Staphylococcus the commander (Once Upon a Time... Life)

Types of pathogen

- **Primary pathogen** – will cause disease in healthy host
  - Presence in body=infection
  - Disease is essential for spread of the pathogen
    - *Treponema pallidum* (syphilis)*, Neisseria gonorrhoeae* (gonorrhoea)*, Mycobacterium tuberculosis* (TBC, consumption), *Yersinia pestis* (plague)
    - relies on the host for survival and spread
  - Accidental pathogen – reservoir outside of the host body
    - *Clostridium tetanii, Bacillus anthracis* – soil bacteria
    - *Legionella pneumophila* – water bacteria

- **Opportunnistic** pathogen – disease in weakend hosts (immunodeficiency, microbiome perturbance, injury etc.)
  - *E. coli, S. aureus*, and many more
  - Usually resident microbiota, presence does not mean infection

Virulence factors

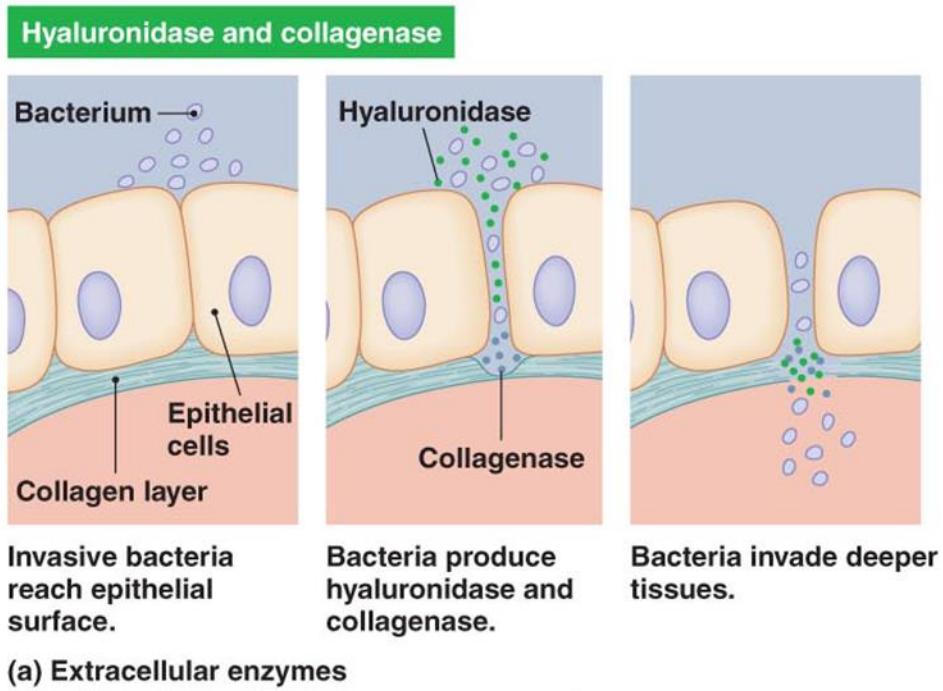
- Allows bacteria to cause a disease

Could be divided

- From the perspective of the host
  - **Invasivity** – adhesion and colonisation
  - **Toxicity** – direct damage to host tissues
  - **Immune dependent factors** – the damage is mediated by the reaction of the immune system

Invasivity

- Adhesion – attachment to the epithelia (adhesins, lipoteichoic acid, capsule)
- Invasion – pentration of the epithelium or into the host cell
  - Enzymes – destruction of extracell matrix – hyaluronidase, colagenase, elastase
  - Invasins – phagocytosis by non profesional phagocytes (e.g. epitelia)
  - Flagellum – penetration through the mucus or epitelia



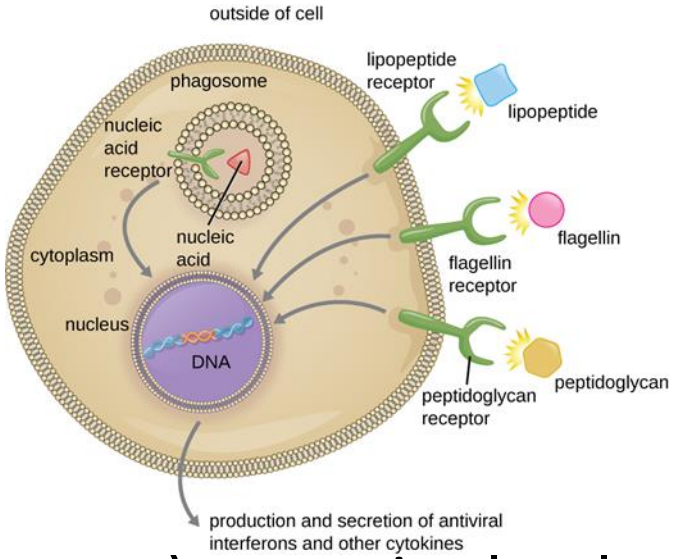
Toxicity

- Damage by direct action or through immune reaction

- Endotoxins
- Exotoxins
- Bacterial metabolites

Endotoxins

- Part of bacterial cell – release after its disintegration
- **P**athogen-**A**ssociated **M**olecular **P**attern (PAMP)
- Molecules recognised by innate immunity (complement and macrophages) as foreign
  - **<u>Lipopolysacharide</u>**
  - Peptidoglycan
  - Teichoic and lipoteichoic acid
  - Flagellum
  - Porins
  - Bacterial DNA (methylation)



➢Cause massive immune response (cytokin storm) – septic shock

Exotoxins

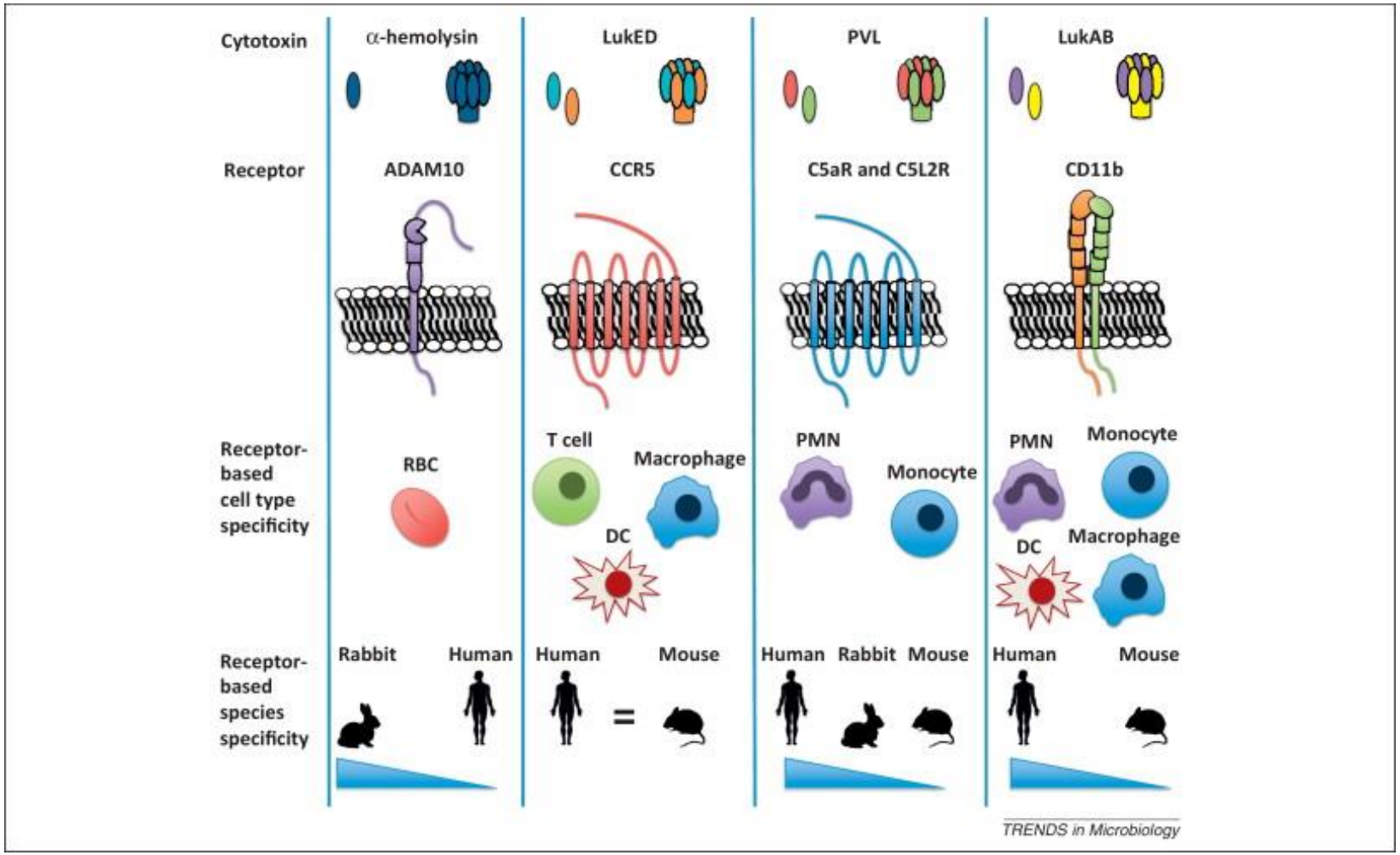
- = „true“ toxins
- Extracelullar **protein** moleculles (compare to endotoxin – outer membrane G-)
- **Direct and serious damage to host**
- Diferentiation – variable criteria:
  - chemical structure (single molecule or macromolecullar complex)
  - Targett structure (cell surface or intracellular)
  - Mechanism (**neurotoxic, enterotoxic**, **cytotoxic**)

Cytotoxins

Pore forming toxins
Mechanism of action

1. Subunits recognise receptor
2. Polymerisation of subunits
3. Insertion of the pore into the mebrane
4. Ion leakage
5. Cell lysis

Cytotoxins of *S. aureus*

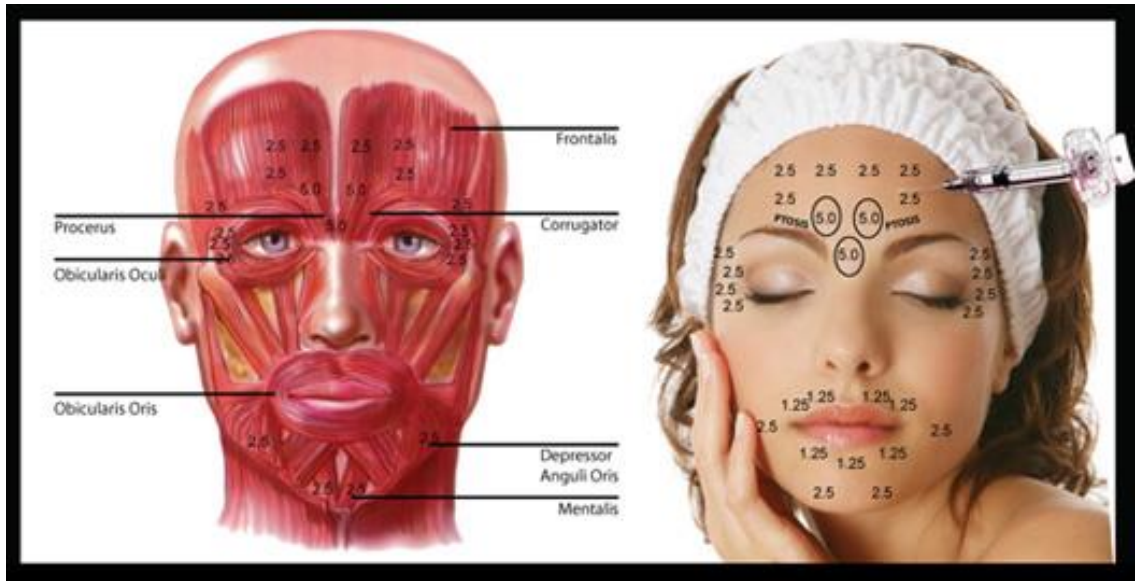


lukED, luk AB, PVL - leukocidins

Neurotoxins

- Extremely low lethal dose
- Produced by clostridia
  - sporulating
  - anaerobic

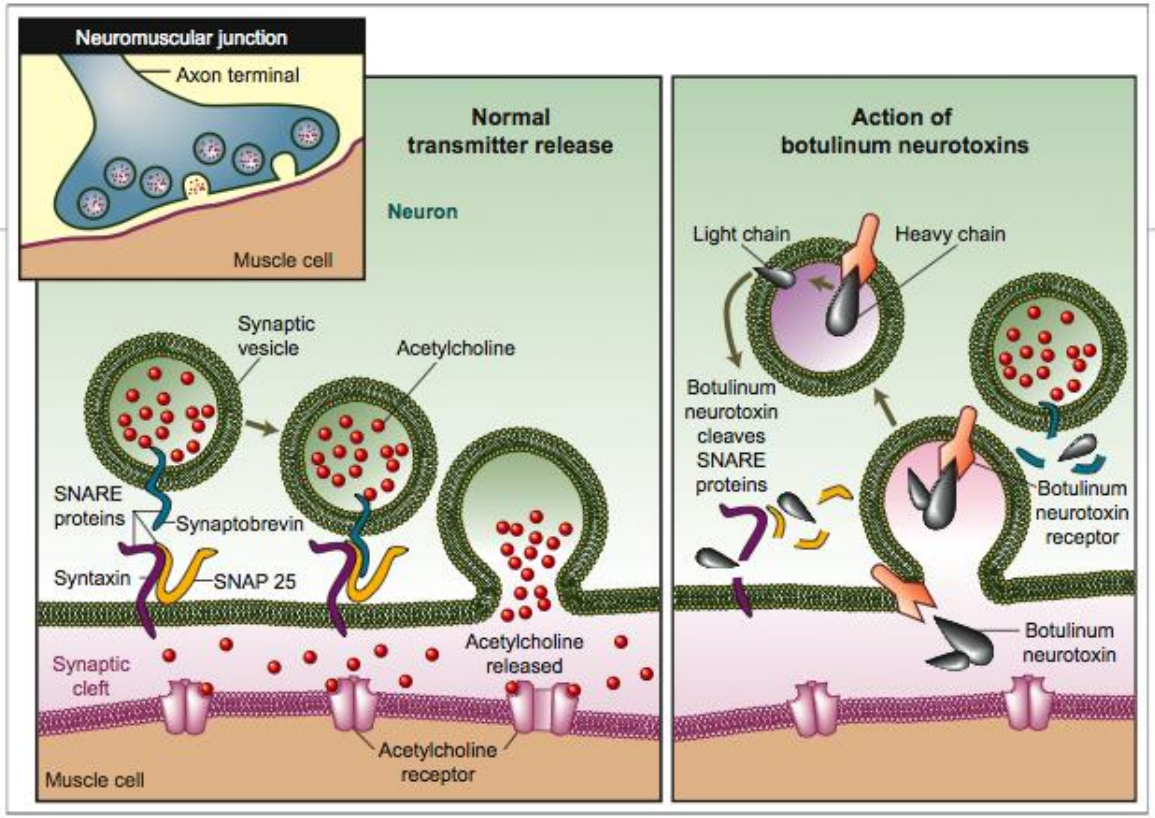
1. botulotoxin



2. tetanospasmin

Neurotoxins

- Mechanism of action: inhibition of fusion of synaptic vesiculles with presynaptic membrane
  - botulotoxin: inhibits acetylcholin release → muscle relaxation
  - tetanospasmin: inhibits GABA and glycine release→ muscle contraction
  - **Disruption of respiration**



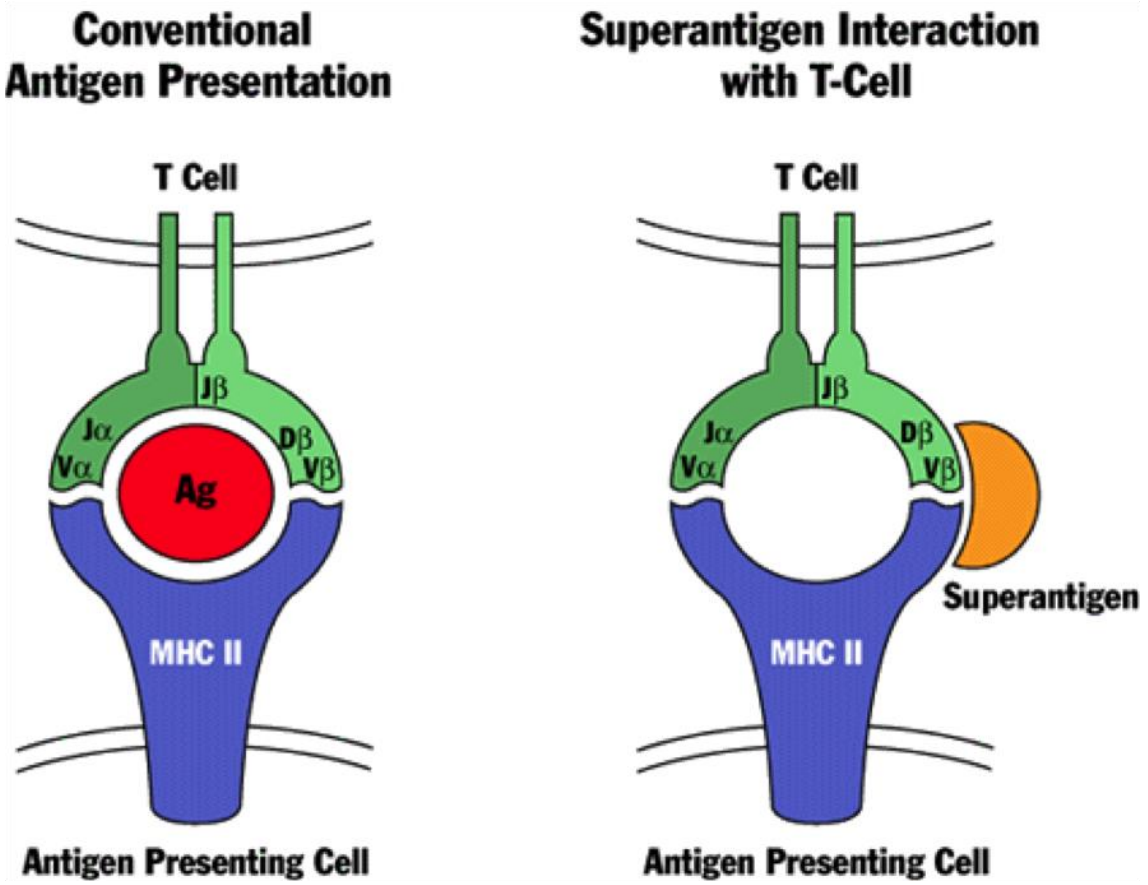
Enteric toxins

- Many patogenic bacteria
  - *E. coli, Shigella, Salmonella, Vibrio cholerae, Campylobacter, Clostridium difficile, S. aureus*
- Poissoning of the *intestinal* epithelium→ **diarrhoea**
  - Typical symptom of intestinal infection
- Diarrhoea
  - patogen: rapid spread in host population
    - Lots of liquid stool filled with bacteria
  - host: cleaning of the intestines

Superantigens

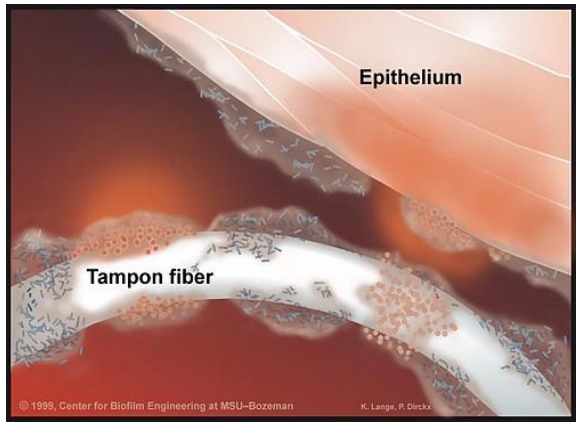
- **super**antigens
  - Direct cross connection between MHC II and TCR
  - **Without specific antigen**
  - **Masive activation of T-lymphocytes**

Examples:
Pyrogenic exotoxins of *Streptococcus pyogenes*
Toxin shock syndrome toxin of *Staphylococcus aureus*
Staphylococcal enterotoxins
Food poissoning



Toxic shock

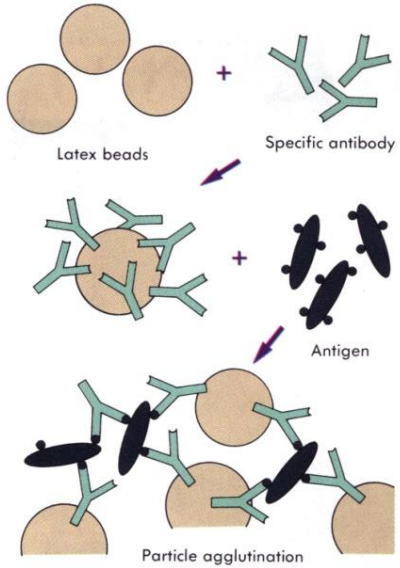
- Caused by superantigens
- TSST-1 of *S. aureus* (less frequently caused by *S. pyogenes*)
- Similar to septic shock
    - Leads to massive cytokine production by activated T-lymfocytes
    - Fever, headache, rash, nauzea diarhoea
    - **Colaps of immune and regulatory homeostasis**
    - **Systemic patological changes**
    - Desquamation after infection
    - CZ 1983-2011 - 159 cases, (47 menstrual form). letality 11 %(staphylococci), 50% (streptococci)

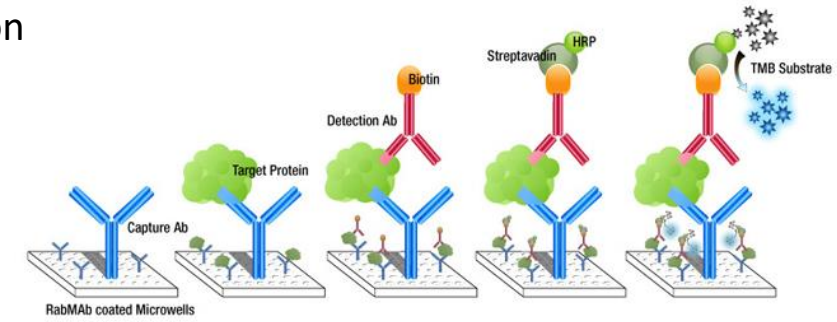


biofilm on tampon fibres reservoir of staphylococci producing toxic shock toxin

Toxin detection

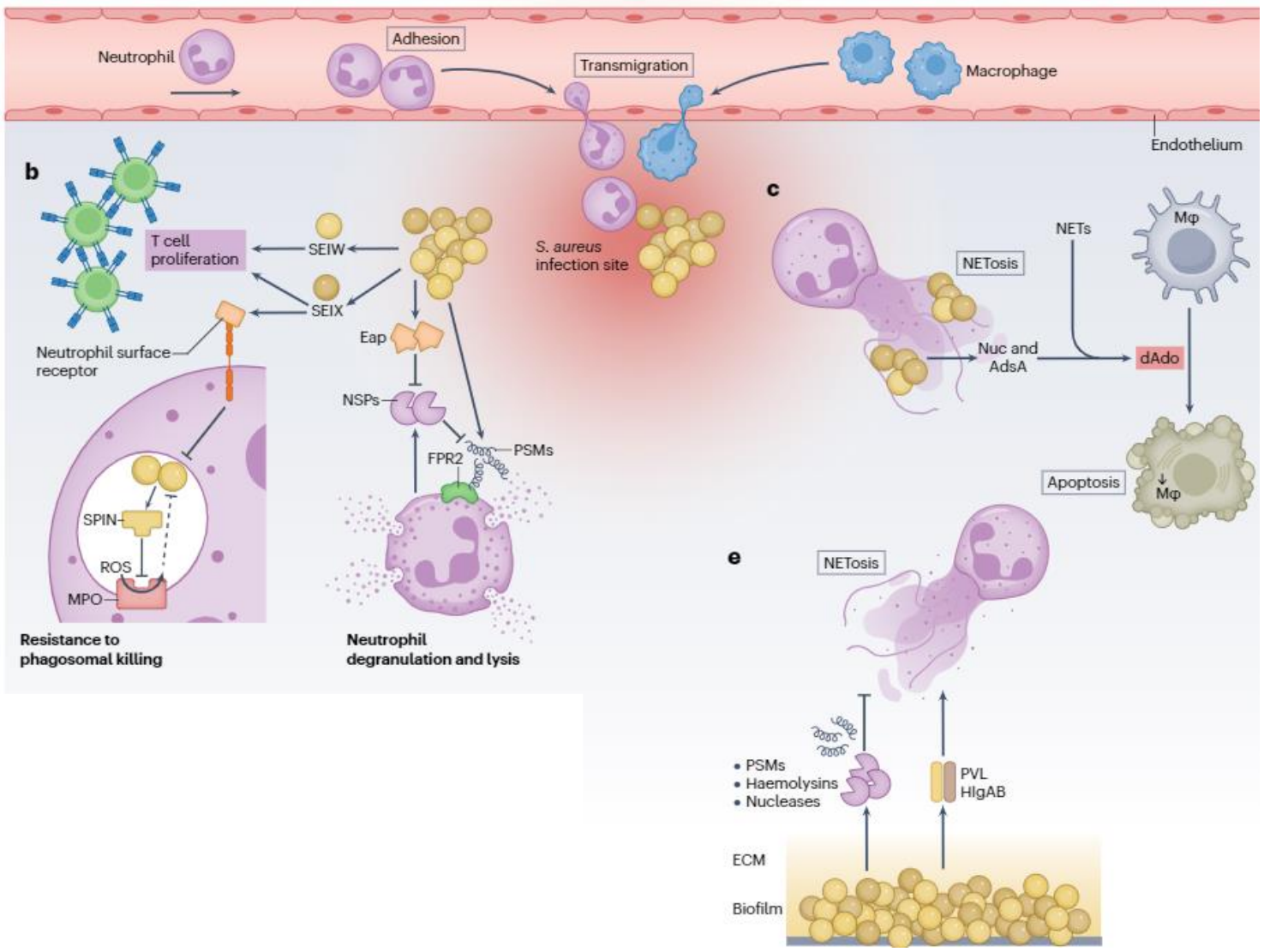
- Detection of toxin moleculles (protein)
  - Specific antibodies
    - latex bead agglutination
    - ELISA
    - Rapid antigen tests





- Detection of toxin genes(DNA)
  - PCR specific for selected genes (primers)

Factors interacting with immmunity



Example of *S. aureus*

Inhibition of neutrophil chemotaxis – CHIPS

Dysregulation of immune response– superantigens (SEA, SEIW, SEIX)

Phagocytosis inhibition –SEIX

Release from NETosis – *nuc* nuclease

Macrophage apoptosis- AdsA

White blood cells lysis– PSM, hemolysins, leukocidins (LukAB, PVL)

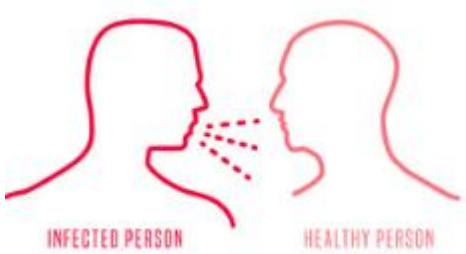
Howden et al, Nature Reviews Microbiology 2023

Why are bacteria pathogenic?

Evolution: the way how to survive and reproduce

Ecology: human body is great place to live – nutrients, temperature, etc

Infection Cycle



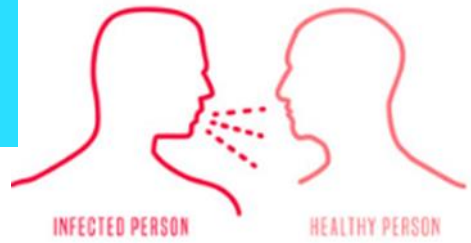
1. Entry of the host

↑virulence

2. Survival in host (defence against IS)

3. reproduction (nutrients exploatation)

↓virulence



4. Transmission (new host)

Before host dies or recover

Bacterial virulence

Hosts viability

Long time interaction leads to optimised virulence

What allows high virulence

Host:
Large population
Frequent connections in population
Sensitive people

High virulence = rapid serious disease with high mortality

Bacteria:
Infection of wrong host (new host, or there is reservoir in another host)
- *Yersinia pestis*

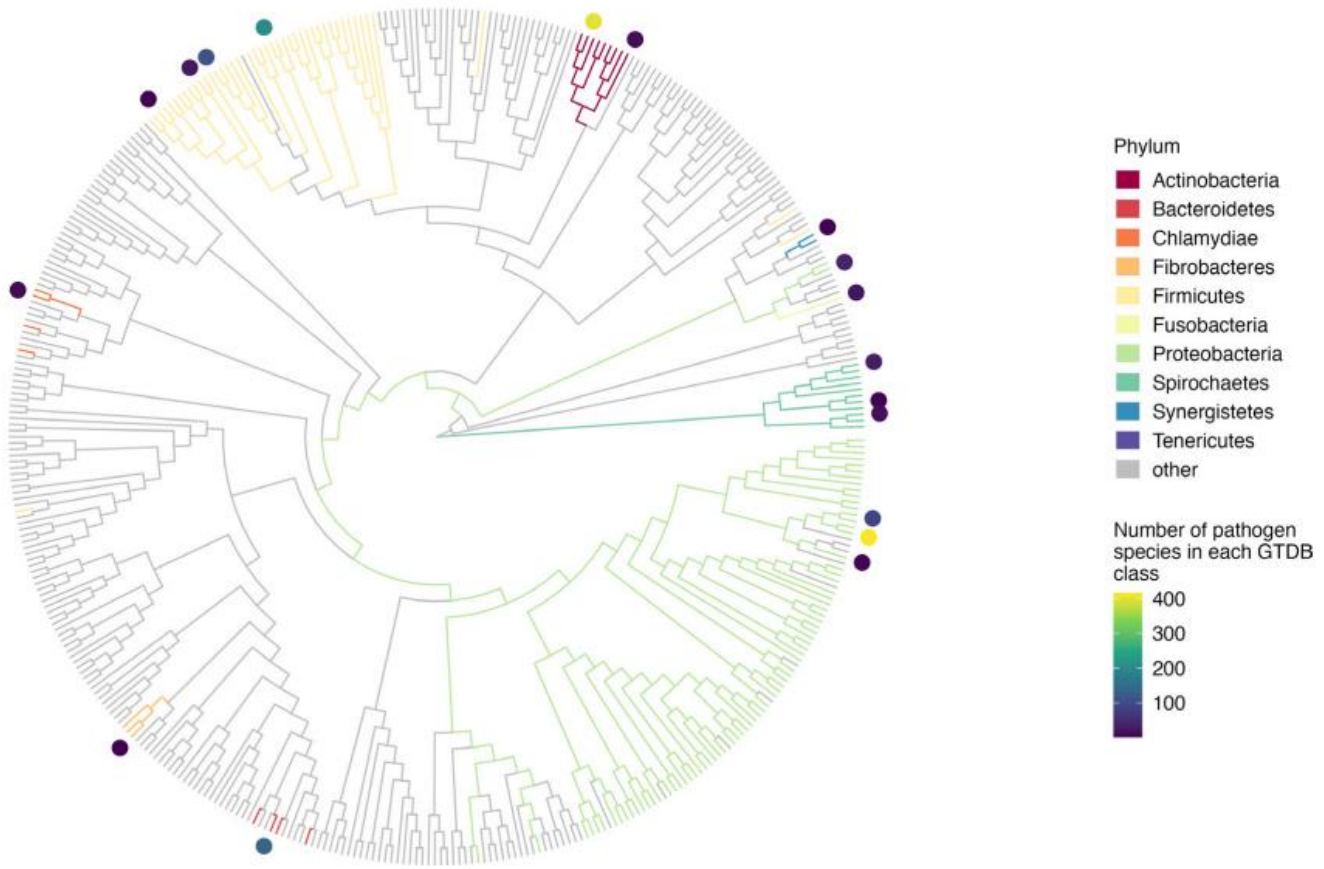
Another survival strategy – e.g. spores, environmental reservoir
- *Bacillus anthracis*
- *Clostridium tetanii* and *Clostridium botulinum*
- *Legionella pneumophila*

Infection of wrong localisation
Staphylococcus aureus:
- colonisation x localised skin infection x systemic infection

How many pathogenic bacteria?

- Bartlett et al., Microbiology 2022
  - 1,531 species of pathogenic bacteria ≈ 7% of bacterial species
  - (Definition of pathogen= at least 3 publication documenting infection)

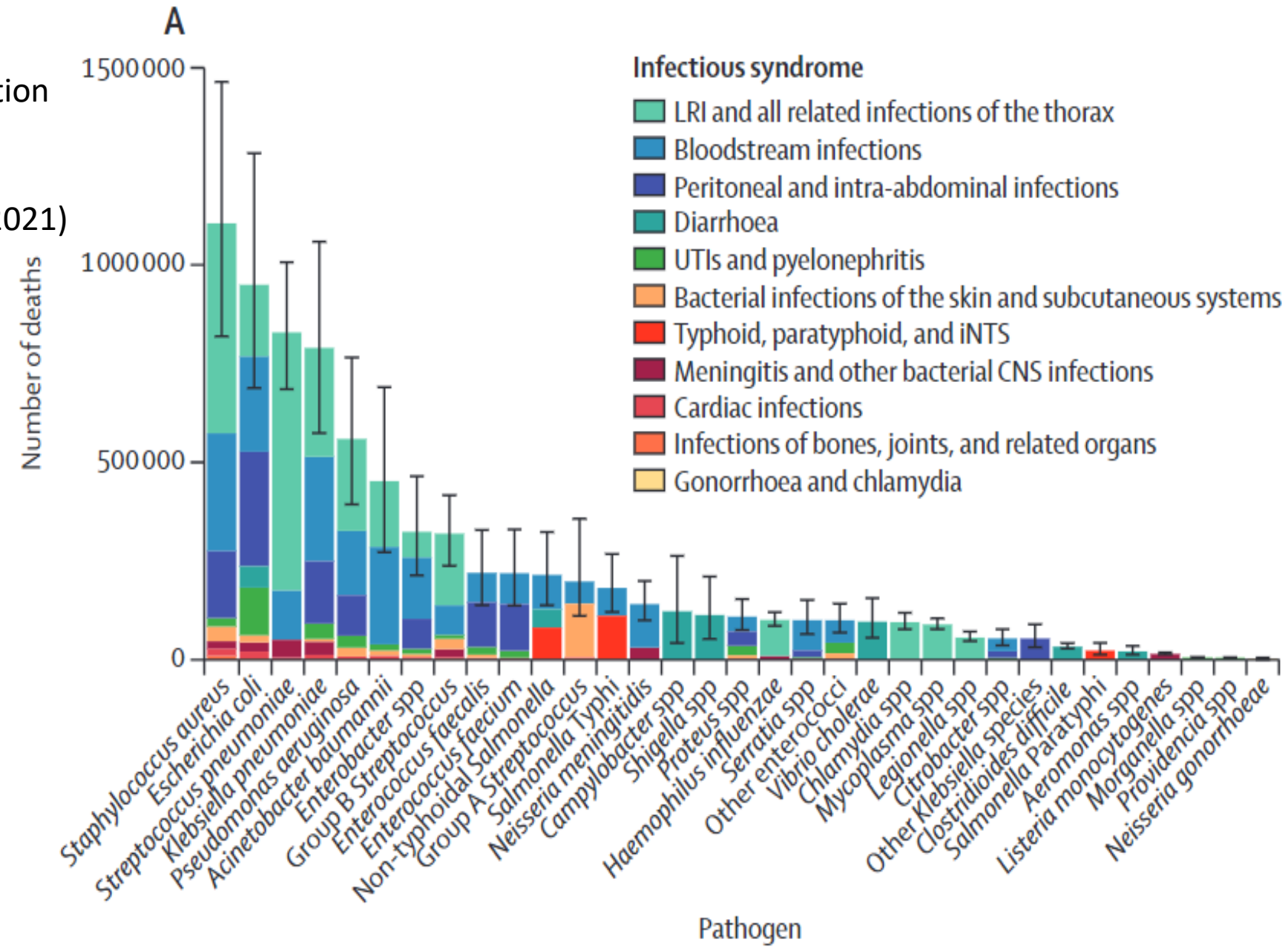
Pathogenic lifestyle is dangerous strategy
Advantage: rapid growth
Disadvantage: host dependency



33 bacterial species
>50% of deaths due to bacterial infection
- (7.7/13.7 mil deaths)

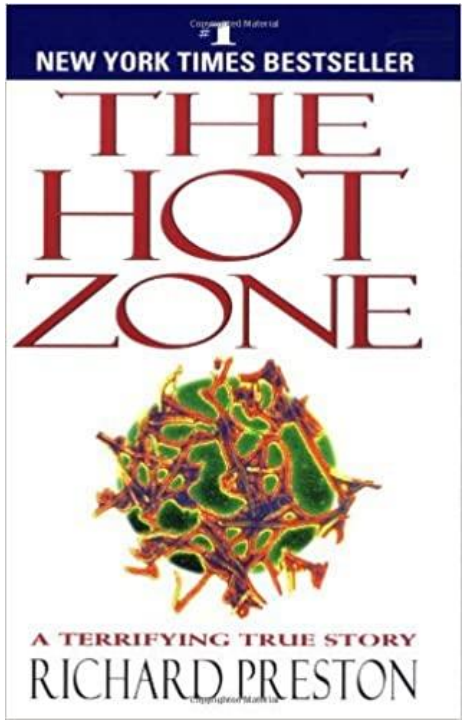
(TBC not included ≈1.6 mil deaths in 2021)

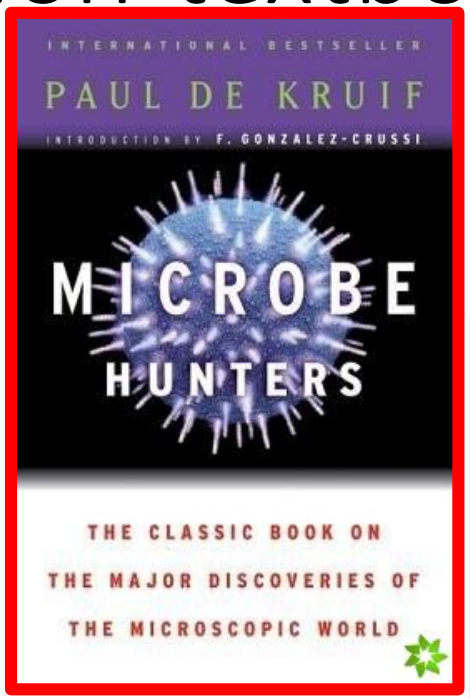
Top:
Staphylococcus aureus
Escherichia coli
Streptococcus pneumoniae
Klebsiella pneumoniae
Pseudomonas aeruginosa

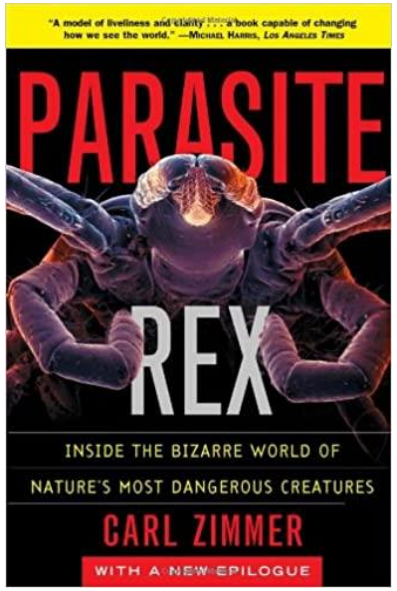


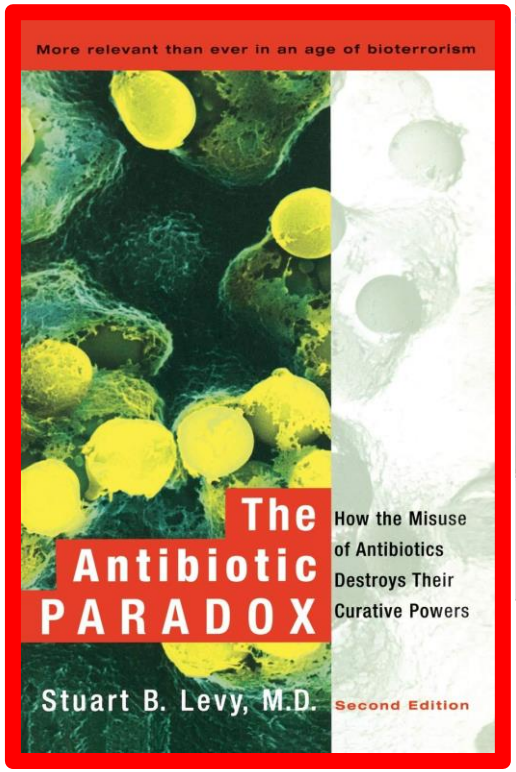
Lancet 2022; 400: 2221–48

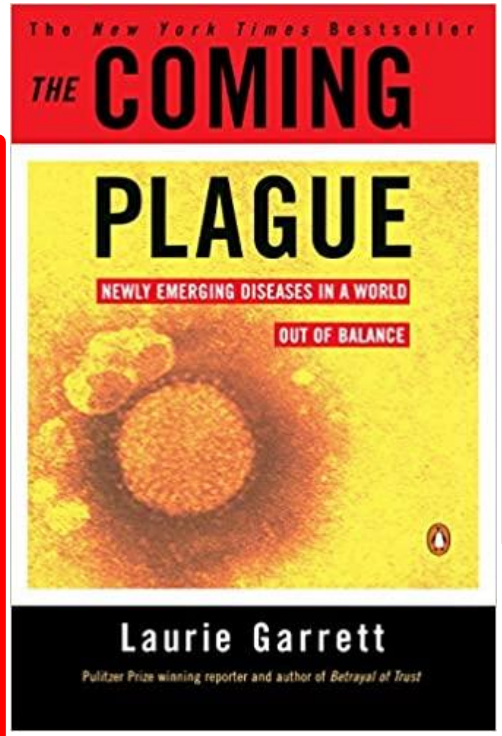
Non-textbook literature

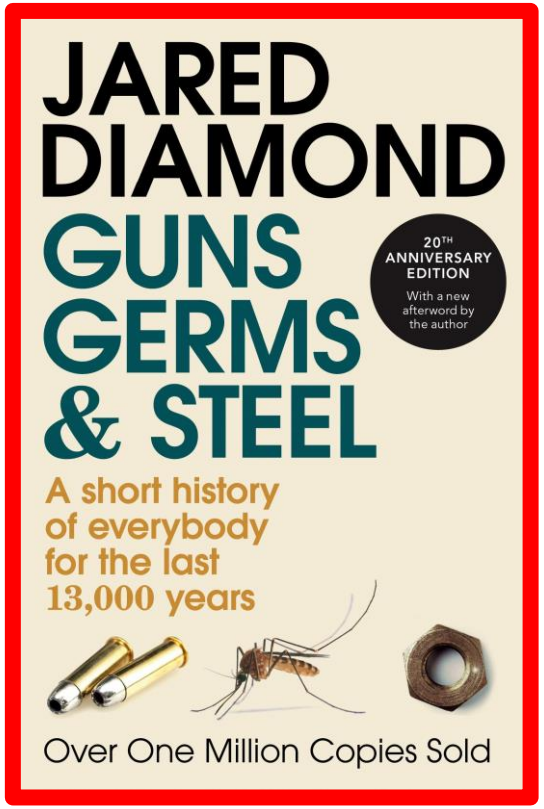












Fine